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STUDIES ON BLACK SCURF OF POTATO
CAUSED BY RHIZOCTONIA SOLANI KUHN

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requirements for the degree of
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SUMMARY

1. The Symptoms of black scurf disease of potato caused by Rhizoctonia solani Kuhn are described and literature relevant to the control of tuber-borne inoculum reviewed.
2. Pathogenic strains of R. solani were present on all 49 certified seed lines screened during 1971 and 1972.
3. Fifteen fungicides varied in their ability to prevent growth of R. solani in poison food tests. An organic mercury compound was the most effective, there being complete suppression of growth at 0.5 ug/ml a.i. Thiabendazole, benomyl, carboxin, chloroneb, thiram and two experimental compounds (BAS 3201F and BAS 3192F) were also highly effective with ED₅₀ values of less than 5 ug/ml a.i.
4. The commercially recommended dipping time and concentration for use with organic mercury compounds inactivated sclerotia. However, the mercury dip treatment as practised by farmers was considerably less effective.
5. Very high concentrations of benomyl (5000 to 7500 ug/ml a.i.) when used as a tuber dip were required to inactivate sclerotia.
6. Increasing dipping time increased the effectiveness of an organic mercury compound but not of benomyl.
7. In glasshouse and field trials benomyl, thiabendazole and carboxin (all 10% a.i.) when applied as tuber dusts gave excellent control of shoot lesioning arising from tuber-borne inoculum. Dip treatment with an organic mercury compound also gave good control. Captan, metiram (both 10% a.i.) and mancozeb (8% a.i.) were less effective but still gave appreciable control.
8. Evidence was obtained indicating that benomyl, thiabendazole, carboxin, metiram, captan and mancozeb when applied as dusts to tubers had a fungistatic effect on sclerotia, whereas an organic mercury dip treatment was fungicidal.
9. Benomyl was systemic in potato plants, the extent of uptake being dependent on the planting medium and the method of application. Uptake was greatest in perlite following

drench application.

10. When benomyl and thiabendazole were applied as tuber dusts uptake was limited to the basal regions of shoots.
11. Benomyl, thiabendazole and carboxin applied as dusts to seed tubers provided some protection against inoculum of R. solani added to the planting medium.

PREFACE

The potato, Solanum tuberosum L. is second only to wheat in importance as a food crop in New Zealand. During the last five years the total area of potatoes grown has fluctuated between 8,000 and 10,000 hectares, and the yield has increased steadily to over 25 tonnes per hectare (Table I).

Table I Potato production in New Zealand. Area and production for several recent seasons*

Season	Area (ha)	Total Yield (tonnes)	Yield (tonnes/ha)
1966-67	8,020	187,267	23.35
1967-68	9,517	235,831	24.78
1968-69	10,132	256,542	25.32
1969-70	9,928	253,263	25.51**
1970-71	7,689 (est.)		

* New Zealand Official Year Book, 1972. (Yield figures converted to metric equivalents).

** = 10.15 tons/acre

Although potatoes are grown in all parts of New Zealand, the bulk of the market is supplied from three areas. The Pukekohe district supplies the early markets from September to December and also substantial quantities of mid-season and main-crop potatoes. The February to July market is supplied principally from the Manawatu-Rangitikei district, and winter supplies (May to November) are drawn mainly from Canterbury, Otago and Southland (Claridge, 1972; Baxter, 1972).

The bulk of seed potato requirements are provided by South Island growers, but increasing quantities are being produced in the North Island on properties situated at elevations greater than 305 metres (1,000 feet) above sea level.

Although many potato varieties are grown in this country, according to an estimate made for the 1967-68 season six varieties accounted for 89% of the total area (Claridge, 1972). Figures extracted from the 1971-72 list of growers of provisionally certified crops (Miller and Wilson, 1972) indicate much the same situation today (Table II).

Table II The relative importance of the main potato varieties grown in New Zealand.

Variety	Percentage of total area occupied by each variety	Percentage of area grown for certified seed occupied by each variety
	1967-68	1971-72
Ilam Hardy	35	45
Aucklander Short Top	14	6
Sebago	12	7
Rua	11	18
Katahdin	10	6
Red King Edward	7	6
Total	89	88

Driver (1963) considers the following to be the most important diseases of the potato in New Zealand.

- (1) Late blight (Phytophthora infestans (Mont.) de Bary).
- (2) Early blight (Alternaria solani (Ell. and Mart.) Sorauer).
- (3) Wilt (Verticillium albo-atrum Reinke and Berthold; Verticillium dahliae Kleb.)
- (4) Blackleg (Erwinia aroideae (Townsd.) Holland; Erwinia atroseptica (van Hall) Jennison; Erwinia carotovora (Jones) Holland)
- (5) Black scurf (Rhizoctonia solani Kuhn)*

* Although the existence of a perfect stage of this fungus has been known since 1903, the mycelial (Rhizoctonia) stage is predominant and for the purposes of this thesis the fungus causing black scurf of potatoes will be referred to as Rhizoctonia solani. The perfect stage is a basidiomycete, Thanatephorus cucumeris (Frank) Donk.

- (6) Scab (Streptomyces scabies (Thaxt.) Waksman and Henrici)
- (7) Powdery scab (Spongospora subterranea (Wallr.) Lagerh.)
- (8) Pinkrot (Phytophthora erythroseptica Pethybr.)
- (9) Virus diseases (leafroll, viruses X, Y, A, S and M).

Of the above diseases, only five are considered by growers to be of sufficient significance to warrant control measures. Control of late blight is based on extensive protective fungicide spray programmes, and the virus diseases are kept in check by planting certified seed and the use of systemic insecticides. By using certified seed, some degree of control of blackleg and wilt is obtained since the certification scheme also takes these diseases into account.

In the belief that shoot infection arising from tuber-borne sclerotia of R. solani significantly affects stand establishment many growers take steps to control this phase of the black scurf disease. Control in recent years has depended almost entirely on the dipping of seed tubers in solutions of organic mercury compounds to inactivate sclerotia. Because of the persistent nature and high mammalian toxicity of these mercury fungicides the Agricultural Chemicals Board in October 1971 advised that use of such chemicals will be banned after two years (J.D. August, pers. comm.). This announcement has had the effect of stimulating considerable interest in alternative tuber treatments to control black scurf.

The present study relates to the seed-borne nature of black scurf and control of tuber-borne inoculum by seed treatments with alternative chemical compounds.

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Chapter I

INTRODUCTION

Black scurf of potatoes, a disease of world-wide distribution (Walker, 1952), is also known by a great variety of other common names, e.g. scurf, black speck, stem canker, little potato disease, Rhizoctonia disease, black scab (Dana, 1925; Heald, 1926) and Corticium disease (in reference to the generic name earlier applied to the perfect stage of the fungus). In the present study "black scurf" is the preferred name as this is the one most commonly applied in New Zealand, and aptly describes the evidence available to growers that inoculum is associated with a particular seed line.

On account of its ready transmission by way of seed tubers, black scurf was probably present in New Zealand from the time of the first introduction of the potato. However, it was not officially recorded until 1922 (Cunningham, 1922) and currently is widely distributed throughout New Zealand (Claridge, 1972). In spite of its prevalence and the widely held belief of its economic significance there have been no serious studies on the disease in New Zealand since those of Cunningham (1925a, 1925b), Cunningham and Neill (1926) and Chamberlain (1931a, 1931b, 1932a, 1932b, 1935).

The following review aims at summarizing salient features of the disease cycle and other information considered relevant to the objectives of the present study.

A. SYMPTOMS

Black scurf presents a great variety of symptoms and signs which may be expressed on shoots, stems, stolons, roots, tubers and in the aerial parts of the plant.

- (i) Shoots and Stems: When seed tubers sprout the growing tip is particularly susceptible to Rhizoctonia attack which is manifest as a reddish-brown

dry necrosis, which in turn induces shoot death. In such cases dormant buds may grow out from below the necrotic area or from the base of the original shoot to form secondary shoots (Figure 1). Depending on the prevailing soil conditions so these secondary shoots may or may not escape infection. Lesions may also develop along shoots as slightly sunken, reddish-brown, elongate necrotic areas which in extreme cases may girdle the stem (Figure 2). It is this conspicuous phase of the disease which arouses the concern of potato growers.

In extreme cases where shoots fail to reach the soil surface the effect is one of "gappiness" in potato crops (Figure 3). In less severe cases where secondary and subsequent shoots develop the effect is that of delayed and uneven emergence. When cankers completely girdle stems immediately below ground level, the normal downward flow of carbohydrates to the developing tubers is disturbed, and their accumulation in the tops is accompanied by stunting, rosetting, formation of aerial tubers in axes of branches and petioles, and enhancement of purpling due to excessive anthocyanin formation (Walker, 1952).

- (ii) Stolons and Roots: Reddish to dark-brown lesions similar to those occurring on shoots are frequently found on stolons. The tips of stolons can be attacked (Figure 4), thereby preventing the formation of tubers, or stolons can be girdled at a later stage resulting in the production of undersized potatoes. A consequence of death of some stolons on the one plant may be the production of oversized tubers on the surviving stolons, due to reduced competition for nutrients.

Root damage is said to be an important phase of the disease, especially under dry conditions where the formation of new roots is retarded (Dana, 1925). Yellowing and rolling of leaves may accompany such damage (Dana, 1925) and it is claimed by Gussow (1917) that the death of fine feeding roots, rather than stem lesions, account for the secondary symptoms in the aerial parts of plants.

(iii) Tubers: Various tuber defects associated with R. solani have been described:

- (a) tuber pits (Morse and Shapovalov, 1914; Ramsey, 1917);
- (b) tuber rots (Shapovalov, 1922);
- (c) irregular, malformed tubers and surface russetting (Morse and Shapovalov, 1924; Dana, 1925).

However, doubt has been expressed as to whether R. solani is the primary pathogen causing tuber pits and irregular malformed tubers (Heald, 1926).

A secondary consequence of damage to both stems and stolons is the formation of "little potatoes" in clusters around the base of stems, or directly from the seed tuber on short stolons (Dana, 1925). The "little potato" condition is illustrated in Figure 5.

It should be noted that some of the symptoms attributed to this disease, particularly aerial symptoms and "little potatoes" can arise from other sources. For example virus diseases may induce rosetting, stunting, yellowing and leaf rolling (Dana, 1925), and the "little potato" condition may result from the use of old or poorly stored seed tubers (Driver, 1972). Further, Edson and Shapovalov (1918) produced stem lesions on potatoes similar to those caused by R. solani by inoculating with several other species of fungi. Throughout the present study isolations were made in all instances where the cause of stem, stolon and root lesions was in doubt. Isolation and identification procedures are detailed in Appendix II.

B. SIGNS

Dark brown or black sclerotia on tubers are the most characteristic and diagnostic signs of the disease (Figure 6). Such sclerotia are irregular in size and shape ranging from minute specks to large angular aggregates an inch or more across. They are entirely superficial but are firmly attached to the tuber surface and leave no perceptible scar when

removed. Dark brown runner hyphae of the fungus can be seen on the tuber surface connecting sclerotia, which are often located very close to eyes (Figure 7) and may even form over eyes.

Frequently a conspicuous brown mantle of interlacing hyphae forms on shoots, roots and stolons (Figure 8). Such mycelium may be abundant without causing any definite lesions, while in other cases lesions may be evident, and the mycelium relatively inconspicuous. Sclerotia are frequently formed from these runner hyphae on shoots, roots and stolons (Figures 1 and 8).

Under warm, moist, conditions, mycelium may aggregate around stem bases to form a whitish weft or mantle (hymenium) which bears the perfect stage of the fungus.



Figure 1 Potato shoot symptoms caused by R. solani, showing the development of secondary shoots following death of primary shoots.



Figure 2 Girdling of potato shoots, caused by R. solani.



Figure 3 "Gappiness" in a commercial potato crop as a result of death of shoots caused by R. solani.



Figure 4 Death of stolon tips, caused by R. solani.



Figure 5 The "little potato" condition.
Note lesions on shoots, caused by R. solani.

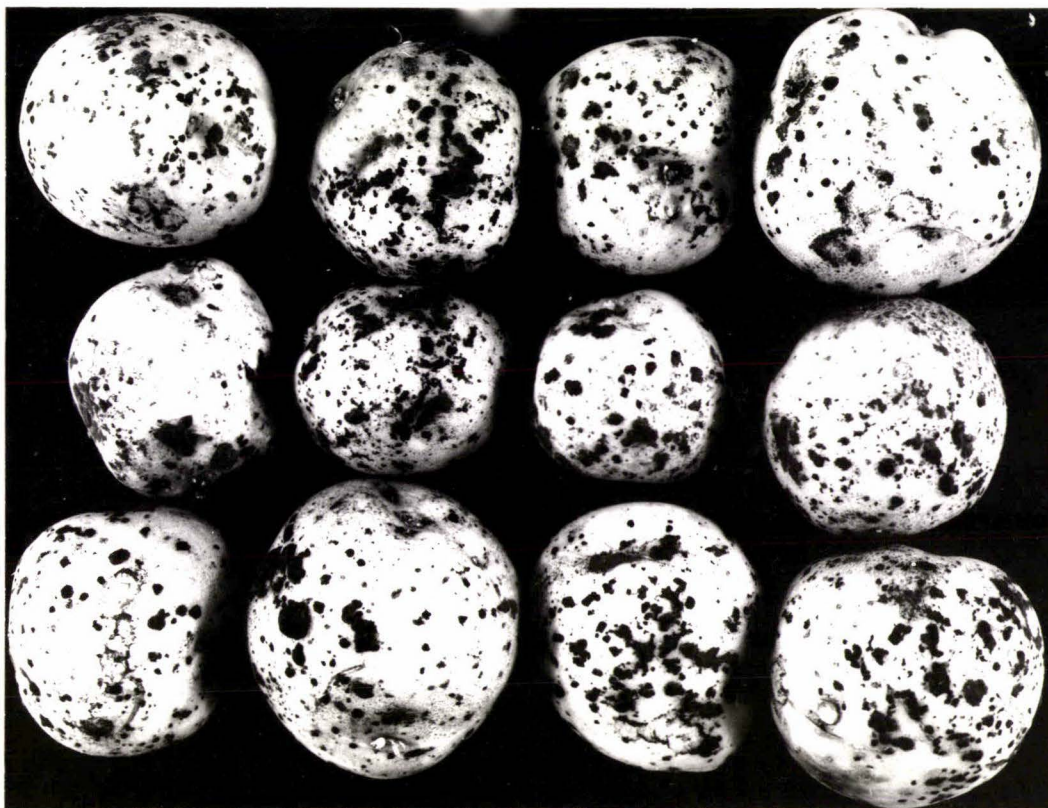


Figure 6 Sclerotia of R. solani on seed tubers
(var. Ilam Hardy, certified seed).

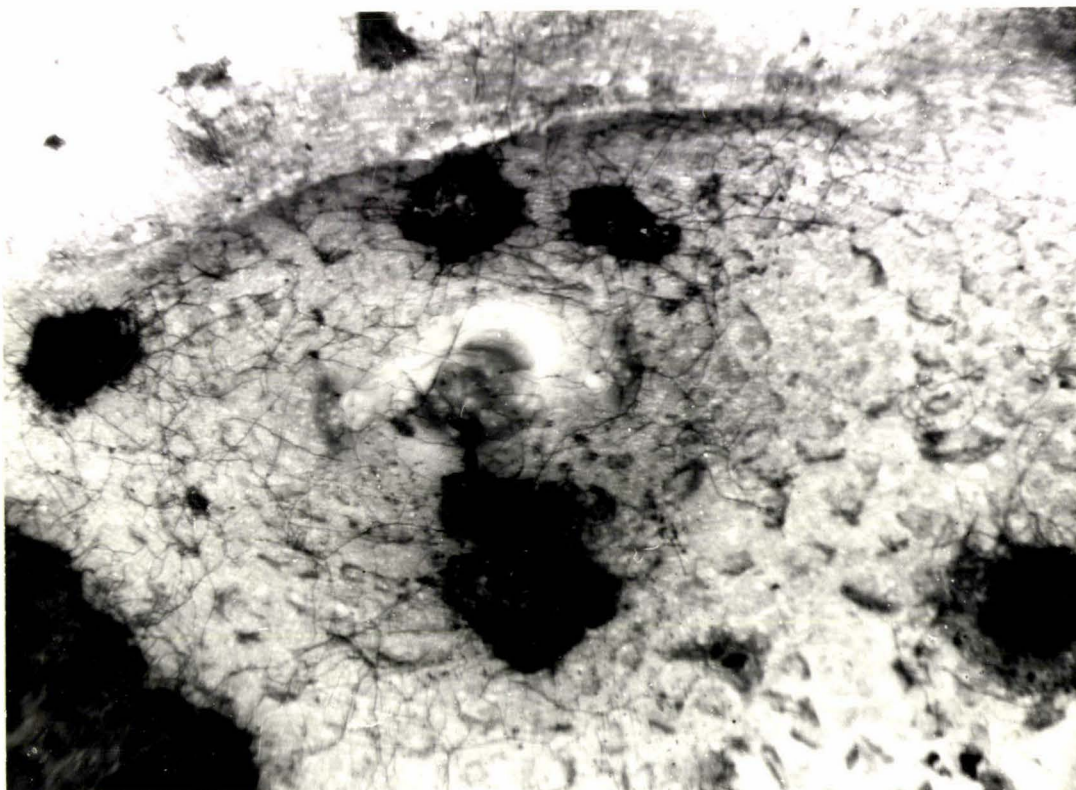


Figure 7 Close-up view of tuber surface showing
proximity of sclerotia and hyphae of
R. solani to eyes.



Figure 8 Runner hyphae and a sclerotium of R. solani on a potato shoot.

C. ETIOLOGY

Primary infections may arise from either of two sources - the soil, or sclerotia adhering to seed tubers (Heald, 1926; Butler and Jones, 1949; Walker, 1952). Pathogenic strains of the fungus may be present in soil as a result of planting infected tubers and conceivably may survive there in three ways:

- (i) As a saprophyte. The competitive saprophytic ability of R. solani has been established (Blair, 1943; Papavizas and Davey, 1962).
- (ii) As an active parasite on other crop or weed hosts. R. solani has a very wide host range (Baker, 1970).
- (iii) In a dormant state. The pathogen could well persist either as dislodged sclerotia, or as sclerotia associated with crop debris.

It is questionable whether strains of R. solani pathogenic to potatoes are present in virgin soils. Pratt (1918) in the United States obtained sclerotia on progeny tubers from sclerotia-free, treated seed and also isolated R. solani directly from soil. However, he did not conduct any pathogenicity tests. Thornton (1958), in New Zealand, isolated R. solani from virgin soils. Although, in the experiments of Morrison, Ross and Thornton (1959), these isolates were not pathogenic to potatoes, no isolate of known pathogenicity was included as a control and so these results are questionable.

There are differing opinions as to the relative importance of seed and soil inoculum, although few definitive experiments have been conducted.

In the United States the experiments of Coons (1918), Melhus and Gilman (1921), Schultz, Gratz and Bond (1930), Schaal (1935) and Raleigh and Bond (1936) indicate that the inoculum potential of R. solani in their soils was very low and that tuber inoculum could be an effective source of the disease. In other experiments in the same country, however, the soil inoculum load has been so high that seed treatments have been of little value (Bisby, Higham and Groh, 1923; Clayton, 1929; Dana, 1925).

Experiments in Canada (Sanford, 1936) to determine the effect of potato tuber treatment on stem infection showed that one-third of the lesions which developed from planting non-treated tubers could be traced to soil inoculum, although great variability existed between individual experiments. Sanford's studies involved 34 experiments conducted over three years under a wide range of field conditions which included different crop sequences, culture and soil types.

In the Netherlands, tuber-borne inoculum is considered the most important source of infection (Emden, Labruyere and Tichelaar, 1966) and all tubers destined for seed purposes are treated in that country (Ir. Th. de Bruin, pers. comm.).

Hide, Hirst and Griffith (1969) state that black scurf may as often, or even more often, arise from soil-borne inoculum as that carried on tubers. However, their surveys of the health status of seed tubers revealed sclerotia of R. solani to be present in numbers considered sufficient to warrant seed treatments (Hirst, Hide, Griffith and Stedman, 1970). In Scotland it is not known how much infection is attributable to soil-borne inoculum under their conditions (A.E.W. Boyd, pers. comm.).

In Victoria (Australia) tuber-borne inoculum is regarded as an important source of infection - probably at least as important as soil-borne inoculum, but the relative importance varies from district to district (D.E. Harrison, pers. comm.).

Experiments in New Zealand indicated that R. solani in the soil can give rise to sclerotia on progeny tubers (Chamberlain, 1931b, 1935). However, the pathogenicity of these strains was not established.

One would be inclined to accept seed-tubers as the more important source of inoculum but for the fact that several workers have shown only a small percentage of isolates from sclerotia on tubers to be pathogenic to potatoes. Sanford (1938a) assigned 20-50% of 114 isolates from random sclerotia on random tubers from four fields to the zero or marginal classes of pathogenic rank. In Australia, tests with sclerotial isolates from a large number of tubers indicated that less than 10% were pathogenic to solanaceous hosts (Flentje, 1967). Person (1945) demonstrated that 70% of 70 sclerotial isolates from potatoes from

six States were either non-pathogenic or only slightly pathogenic, and only 7% were strongly pathogenic.

Basidiospores are commonly produced in potato crops, but the part they play in the disease cycle is obscure. It is stated, without evidence, that basidiospores can germinate to establish mycelium in the soil (Heald, 1926) and since basidiospore isolates have proved to be pathogenic to potato stems (Sanford, 1938a) it is conceivable that air-borne basidiospores may function to increase the soil inoculum load.

D. EPIDEMIOLOGY

Many soil factors, temperature, moisture, aeration, pH, texture, structure, nutrient status and microbial interactions may influence the development of black scurf, and of these, temperature and moisture are considered the most important (Heald, 1926; Butler and Jones, 1949).

Richards (1921), using Wisconsin soil temperature tanks, unsterilized soil and naturally infected tubers, demonstrated that lower soil temperatures were most conducive to the development of stem lesions. He obtained lesions on stems at temperatures between 9C and 27C, with the most severe attack occurring in the 12C to 21C range (optimum 18C). However, relatively serious damage frequently occurred at temperatures as low as 9C. Richards further observed that the destruction of growing points was most severe between 12C and 18C, but negligible at and above 21C. He also found that at 24C potato shoots emerged in about one-third of the time it took at 15C and this fact he suggested provided one explanation for the "escape" of shoots from injury at higher temperatures and the ability of later-formed secondary shoots to escape infection.

Richards (1923) also conducted field experiments involving different planting dates and different years and successfully correlated the prevalence and severity of the disease with lower soil temperatures.

In a series of tests with many isolates each uniformly incorporated in unsterilized soil, Sanford (1938b) was unable to

confirm Richards' results. He concluded that the results obtained in separate tests were not sufficiently definite or consistent to warrant the conclusion that disease expression was greater at 16, 18, 20 or 23C, or that growing point destruction was more severe at the lower temperatures.

This apparent conflict can be explained when one considers the source of infection in the two series of experiments. In Richards' studies, where tuber-borne inoculum was involved, it would seem reasonable for emerging shoots to "outstrip" the pathogen at higher temperatures. By contrast, in Sanford's experiments infections arose from inoculum uniformly distributed throughout the test soil, in which case shoot infections would be more likely to occur irrespective of soil temperature. Perhaps the work of Richards and Sanford allows the generalization that low soil temperature enhances the possibility of shoot infections, irrespective of inoculum source, and that at higher soil temperatures shoot destruction will be minimized if seed tubers are the main inoculum source.

No definite conclusion can be drawn as to the importance of soil moisture levels in governing the extent and severity of black scurf, as apparent from the studies of Sanford (1938b). He noted that the recovery of the host by means of secondary and tertiary shoots was better in a wet soil than in a dry one, regardless of temperature, but was unable to conclude whether a dry soil or a wet soil was more favourable for disease development. However, according to Boyd (1969) and McKay (1955) the disease is more severe under dry conditions, and Clark and Martin (1931) demonstrated that cankering of stems and stolons was much more severe in soils with a 20% moisture content than in those with a higher moisture content. By contrast, Rolfs (1904) found that heavy, poorly drained soils favoured the development of disease, and Schaal (1935) demonstrated that heavy irrigation increased the contamination of tubers with sclerotia.

The growth of R. solani through soil is restricted by poor aeration (Blair, 1943) and since aeration is influenced by soil

moisture, structure and texture, all these factors could possibly affect disease development.

Soil reaction is regarded by Heald (1926) as an important factor influencing the severity of black scurf. He suggests that R. solani thrives best in acid soils and reports that liming can reduce the severity of the disease. Morse and Shapovalov (1916) increased the infection of potato stems by 20% to 30% with row applications of sulphur, which presumably lowered the soil pH. However, McGreary (1967) reduced the incidence of sclerotia on tubers with soil applications of sulphur and increased the incidence with lime applications. Rhizoctonia solani grows through soil over a broad pH range with maximum growth at pH 7.0 (Blair, 1943) and Danish work (Frederiksen, Jorgenson, and Nielsen, 1938) demonstrated that soil pH within the range 4.7 to 7.6 was of little importance in the development of stem lesions. It would seem that pH within the range of most agricultural soils has little influence on the disease, although indirect effects of soil pH on available nutrients and the soil microbial population may be important.

The nutrient status of the soil, both organic and inorganic, will no doubt influence disease development because of effects on either the host, the pathogen or the other soil micro-organisms. Microbial interactions (synergism, antibiosis, hyperparasitism, fungistasis and lysis, competition) will also influence disease development. The effect of nutrition and microbial interactions on Rhizoctonia diseases in general and on the growth of R. solani in soil are considered by Baker and Martinson (1970) and Papavizas (1970).

According to workers in the Netherlands potato shoots become more resistant to attack following emergence (Emden et al., 1966). Thus, in any situation where shoot emergence is delayed, the chances of severe disease developing will increase, all other factors being equal. Factors which could reduce the rate of emergence of shoots include:-

- (i) unfavourable temperature and moisture conditions;
- (ii) reduced vigour of shoots because of virus diseases, or poor storage treatment of seed tubers;

- (iii) planting depth. Stormer and Ebell (1944) obtained over a 50% increase in the number of severely diseased shoots by increasing planting depth from 5 cm to 15 cm. Thus some control of the disease could be obtained by shallow planting of tubers and using subsequent cultivations to form moulds. This would increase the rate of emergence of shoots, firstly because of the shorter distance they have to grow to reach the soil surface, and secondly, because of higher temperatures within this shallower covering.

A common cultural practice used in New Zealand for weed control is to harrow down moulds shortly before the first shoots emerge. It would seem that this practice has been an unconscious method of minimizing the disease by allowing shoots to emerge more quickly.

By contrast, cultural practices associated with use of pre-emergence herbicides enhance the likelihood of shoot infection. Weed control in this manner involves moulding the crop immediately prior to emergence followed by spray application of the herbicide. Since the moulds are subsequently left undisturbed, potato shoots are required to grow up through a considerable depth of soil with greatly increased chances of shoot infection occurring. This would apply more particularly to such varieties as Rua and Sebago which inherently are slow emerging.

E. EXTENT OF LOSSES

Reports as to the loss directly attributable to black scurf are conflicting.

Estimates from the United States between 1928 and 1939 claim an average loss in yield of 2.5%, with up to 10% loss reported from some States (Wood, 1932; McCallan, 1946). Reports of individual experiments conducted in the United States involving seed or soil treatments and/or seed selection indicate losses as either, severe (Cook and Lint, 1916; Richards, 1923), moderate but still significant (Dana, 1925; Leach, Johnson and Parson, 1929; Schultz *et al.*, 1930; Raleigh and Bond, 1936; Livingston, Oshima and Morrill, 1962; Cetas, 1971; Davis, Groskopp and Callihan, 1971), slight (Coons, 1918; Schaal,

1935; Schultz, 1966), or negligible (Heald, 1926; Biehn, 1969; Cetas, 1969a, 1970; Easton, Maxwell, Oldenburg and Anderson, 1970; Harrison, Johnson and Barmington, 1970).

However, the losses reported by some of the above investigators varied from year to year. For example, Richards (1923) obtained a 50% yield increase by seed treatment in 1918 but only a 15% increase in 1919. Likewise, Schultz, et al. (1930) and Raleigh and Bond (1936) obtained yield increases of up to 20% with seed treatments in some years but no increase in other years.

In Canada (Sanford, 1937) extensive experiments failed to show any consistent effects of the disease on total tuber yield, or the yield of large size or small size tubers.

Small (1943, 1945) as a result of experiments conducted over four years, concluded that black scurf causes little, if any, loss under farm conditions in England. He failed to show any reduction in yield despite quite severe attacks on shoots and stolons. Other experiments in the United Kingdom involving seed selected for the extent of infection with sclerotia indicated only slight losses in yield in all instances (Hirst, et al., 1970). Only a 6% to 7% reduction in yield resulted from planting severely infected seed, while moderately infected and unselected seed gave yield reductions of 4% to 5% and 3% respectively.

Australian and Dutch workers consider that the most important effect of black scurf is on the quality (size, shape and colour) of harvested tubers (D.E. Harrison, pers. comm.; Emden et al., 1966). However, significant increases in yield have been obtained by control of seed and soil inoculum in both Australia (D.E. Harrison, pers. comm.) and the Netherlands (Ten Boer, 1958; Lint and Emden, 1957).

In New Zealand the experiments of Cunningham and Neill (1926) using seed treatments, and those of Chamberlain (1932b) involving seed selection and seed treatments failed to show any consistent effect of the disease on yield. Chamberlain concluded that treatment of seed potatoes under New Zealand conditions was not warranted, mainly because of the doubtful effect of black scurf on yield and the possible injurious effects

of the then available seed treatments.

In field trials in the Rangitikei, Booth (1963) obtained increases in the yield of table potatoes of up to 27% following seed treatment. However, there was no evidence provided to prove that this increased yield was in fact due to control of black scurf.

Several factors could account for the variability apparent in the experiments reviewed above.

- (i) In studies involving seed and soil treatments, these treatments themselves could have reduced yields, for example in the experiments of Dana (1925), Chamberlain (1932b) and Sanford (1937).
- (ii) Seed selection may not have been effective in providing inoculum-free seed. Minute sclerotia and mycelium on "clean" tubers could provide sufficient inoculum to confuse results.
- (iii) In experiments involving seed treatments, soil inoculum could have confused the results obtained, and vice versa.
- (iv) In individual tests unsuitable environmental conditions or the absence of pathogenic strains could well have accounted for the absence of any adverse effect on yield.
- (v) Where misses occurred or plants were severely diseased then adjacent plants could have compensated to an extent that yield was not reduced. Hirst et al., (1970) have demonstrated in healthy crops that removing 24% of the plants from random positions at emergence only resulted in a 9% reduction in total yield. They also claim that to obtain a 5% decrease in yield (approximately the least decrease that experimentally can be reliably detected) at least 15% of the plants would have to be removed at emergence.

It must be appreciated that the above percentages relate strictly to the circumstances of their experiments. Prevailing environmental conditions, variety, seed size, within-row and between-row spacing are all factors which in any crop could influence the degree of compensation provided by adjacent plants.

F. DISCUSSION

From the above review it is clear that in spite of considerable research throughout the world, there is little agreement on such basic questions as to the relative importance of soil-borne and tuber-borne inoculum, the influence of specific environmental factors and the economic significance of the disease. This is understandable if one accepts that each planting is in effect a unique situation. In every situation there will be variation in the seed-tuber and/or soil inoculum load, and the extent to which inoculum from either source will cause loss will be dependent in part on the virulence of the strain of R. solani present, and the prevailing soil environmental conditions. Obviously then, there is danger in generalizing on any aspect of the potato black scurf disease. Nevertheless, in the present study one basic assumption is made:

- that in New Zealand tuber-borne inoculum is the more important source of infection.

This view is held for the following reasons:

- (a) In most instances potatoes are grown in soil out of long established pastures. Chamberlain (1935) in this country demonstrated that returning soil to pasture for 3 to 4 years practically eliminated R. solani. It follows that with the exception of those areas where potatoes are continuously cropped (e.g. Pukekohe) the soil-borne inoculum load must inevitably be low or non-existent.
- (b) In a health survey of certified seed potatoes (Appendix III) pathogenic strains of R. solani were present on all lines examined, indicating seed-tubers as a consistent source of inoculum.

An obvious corollary to the above-stated premise is that control of black scurf will be consequent on use of an effective method of inactivating inoculum associated with seed tubers. Since mercury based compounds currently used for dipping seed tubers are to be withdrawn from the New Zealand market later this year (J.D. August, pers. comm.), there is obvious need for assessing the effectiveness of alternative chemical compounds for seed tuber treatment. This has been the major objective of

the present study. Specifically the work has involved:

- (a) Screening a range of fungicides as to their ability to prevent mycelial growth and inactivate sclerotia of R. solani under laboratory conditions.
- (b) Determining the capacity of various fungicides to prevent development of shoot lesions when applied as seed tuber treatments under glasshouse and field conditions.
- (c) Investigations on the systemic properties of some fungicides in relation to prevention of shoot lesioning.

Chapter II

LABORATORY SCREENING OF FUNGICIDES

A. THE POISON FOOD TECHNIQUE

1. Introduction: Many in vitro and in vivo techniques have been developed during the last 40 years that attempt to measure the fungitoxicity of chemicals or compare the fungitoxic values of chemicals. In vitro tests are basically modifications of the so-called spore germination method and the poison food technique, and these have been reviewed by several investigators (Horsfall, 1945, 1956; McCallan, 1959; Torgeson, 1967), more recently by Neely (1969).

In the present study the poison food technique was used; that is, comparisons were made of the radial growth rates of the fungus on substrates impregnated with different concentrations of fungicides. The method was largely developed for work with wood-rotting fungi (Horsfall, 1956), but is equally useful with organisms which either do not sporulate, or cannot readily be induced to do so in the laboratory (Torgeson, 1967). Many other investigators have previously used this technique to screen candidate fungicides against R. solani (Edgington and Barron, 1967; Borum and Sinclair, 1968; Al-Beldawi and Sinclair, 1969; Darrag and Sinclair, 1969; Al-Beldawi and Pinckard, 1970; Snel, von Schmeling and Edgington, 1970; Bollen and Fuchs, 1970; Edgington, Khew and Barron, 1971; Davis and Pinckard, 1971).

2. Materials and Methods: Tests were conducted with 15 fungicides (Table III) using three isolates of R. solani obtained from lesions on potato shoots from three different seed lines.

Each fungicide was tested initially at concentrations of 0, 5, 10, 20, 50, 100 ug/ml active ingredient (a.i.) in PDA_L or PDA_D. Since in this experiment 6 of the 15

Table III Fungicides used in poison food tests.

<u>Common Name or Code No.</u>	<u>Trade Name</u>	<u>Chemical Name and Formulation</u>
carboxin	Vitavax	5, 6-dihydro-2-methyl-1, 4 oxathiin-3-carboxanilide. 10% ^W /w.
oxycarboxin	Plantvax	5, 6-dihydro-2-methyl-1, 4 oxathiin-3-carboxanilide-4, 4 dioxide. 75% ^W /w.
thiabendazole	Mertect, Tecto	2-(4-thiazolyl) benzimidazole 10% ^W /w
fuberidazole		2-(2-furyl) benzimidazole 50% ^W /w
benomyl	Benlate	methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate 50% ^W /w
BAS 3201F	Bavistin	1-($\sqrt{2}$ -(methylthio)ethyl)carbamoyl)-2-(methoxyamino) benzimidazole 50% ^W /w
thiram	Thiram 80	bis(dimethylthiocarbamoyl)disulphide 80% ^W /w
BAS 3192F		2, 5, -dimethyl-furane-3 carbonic acid anilide 50% ^W /w
mancozeb	Dithane M.45	Co-ordination product of zinc ion and maneb (manganous ethylene bis dithiocartanate) 8% ^W /w
metiram	Polyram Combi	A complex of zineb and polyethylene thiuram disulphide. 10% ^W /w
captan	Orthocide	N-((trichloromethyl)thio)-4-cyclohexene-1, 2-dicarboximide 10% ^W /w
	Baytan Forte	2-methoxyethyl mercuric chloride (MEMC) (6% Hg)
chloroneb	Demosan	1, 4-dichloro-2, 5-dimethoxybenzene 65% ^W /w

ctd. over

Table III Fungicides used in poison food tests. (ctd)

<u>Common Name or Code No.</u>	<u>Trade Name</u>	<u>Chemical Name and Formulation</u>
quintozene	Terrachlor	pentachloronitrobenzene (PCNB) 75% ^w /w
ethazol	Terrazole	5-ethoxy-3-trichloromethyl-1, 2, 4-thiadiazole 35% ^w /w

fungicides were completely inhibitory to R. solani at concentrations of 5 or 10 ug/ml a.i., further tests were conducted with these 6 chemicals at 0, 0.5, 1.0, 2.5, 5.0, 7.5, and 10.0 ug/ml a.i.

In preparing the poison food plates with each fungicide, the method of Hor (1972) was used with minor modifications.* Seventy-five millilitres of sterile molten PDA_L or PDA_D were added to each of the required number of sterile flasks (150 ml) and maintained at 55C in a water bath. For the initial tests a stock solution of each fungicide (500 ug/ml a.i.) was prepared by adding the appropriate amount of fungicide to 250 ml of sterile distilled water. For tests involving lower concentrations (0-10.0 ug/ml a.i.) a second stock solution was prepared by diluting a 500 ug/ml a.i. solution to 50 ug/ml a.i. With the aid of a pipette the required volume of fungicide from the stock solution was added to sterile distilled water to obtain 75 ml aliquotes, each double the concentration to be tested. Each was then added to one of the flasks of double strength PDA maintained at 55C. Controls consisted of 75 ml sterile distilled water added to 75 ml double strength PDA. The flasks were shaken to mix the agar and fungicide thoroughly, and the contents poured into nine plastic petri plates at a rate of 16-17 ml/plate. Once cooled the plates were centrally inoculated with a mycelial plug (4 mm diam., mycelial face downwards) removed from four to seven day old test cultures. Three replicates were used for each treatment.

The inoculated plates were incubated in the dark at 24C until control colonies had reached the edge of the petri dishes (approximately 75 hours) at which time the radial growth of each culture was recorded by averaging two measurements at right angles to each other.

Tests were also conducted to determine whether the action of the fungicides was fungistatic or fungicidal. At

* The method is summarized schematically in diagram form in Figure 9.

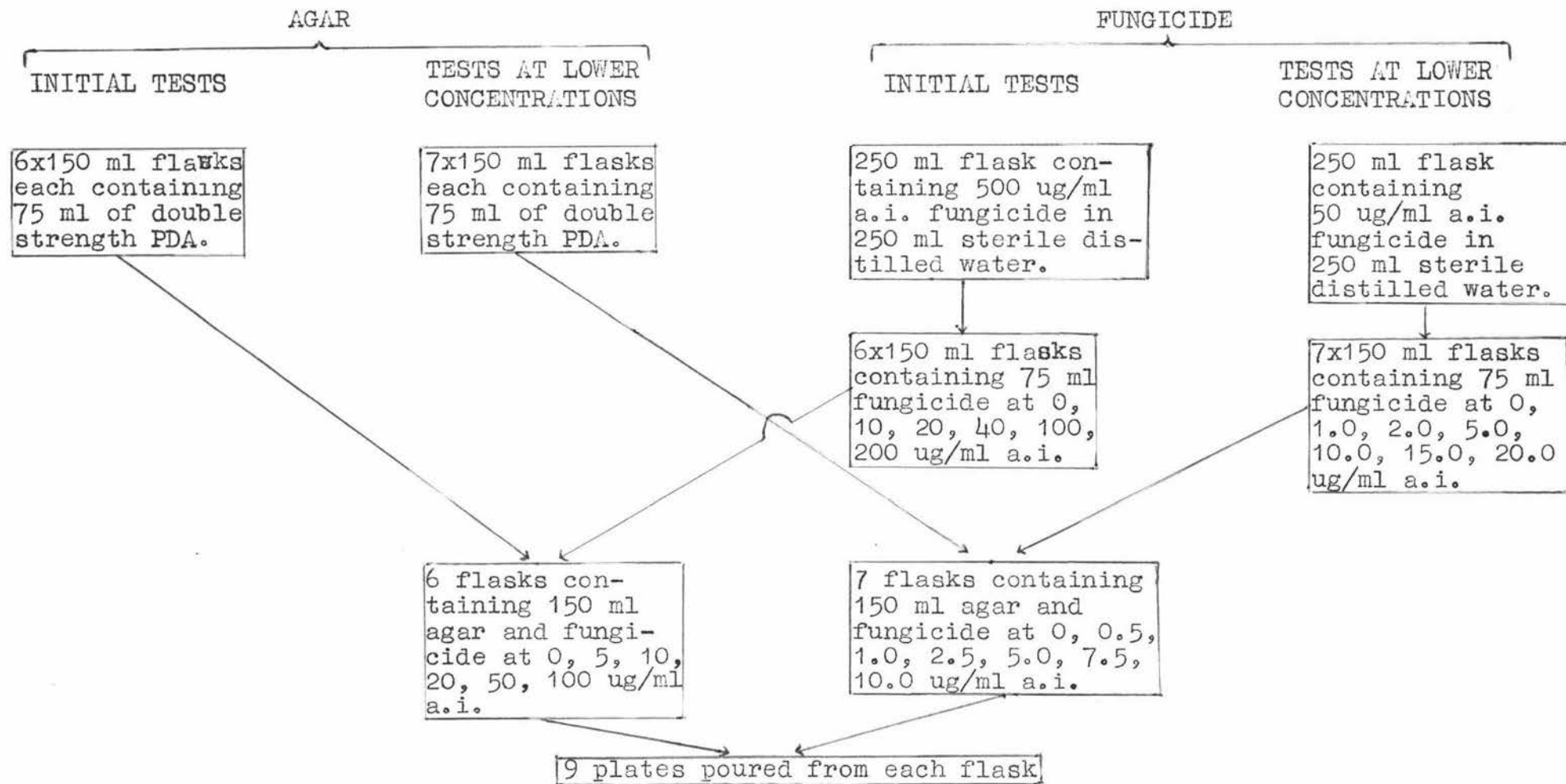


Figure 9 Diagram of procedure used for poison food assay of fungicides.

the end of each experiment mycelial plugs which showed no evidence of growth were transferred to PDA_L plates free of fungicide. The plugs were examined for mycelial growth after three days incubation at 24C. If growth resumed it would indicate that the fungicide was simply inhibiting growth, i.e. fungistatic action. If, however, growth was not evident the fungicide presumably killed the fungus, in which case the action was fungicidal (McCallan and Wellman, 1942).

Fungicides were tested in groups of three since it was not convenient to test all 15 concurrently. Results for each fungicide are expressed as percentage inhibition of radial growth as compared with controls, and since controls were included in each experiment the results from individual tests are comparable.

3. Results and Discussion: The results are illustrated graphically for all fungicides (Figure 10), and photographically for captan, benomyl and thiabendazole (Figures 11 and 12). The fungicides are also ranked, firstly according to their approximate ED₅₀ values (Table IV), and secondly, according to the lowest concentration tested which completely inhibited mycelial growth (Table V).

Table IV Ranking of fungicides according to approximate ED₅₀ values.*

ED ₅₀ (ug/ml a.i.)			
< 5	5-10	10-20	20-50
MEMC	captan	metiram	PCNB
thiabendazole	fuberidazole	mancozeb	oxycarboxin
benomyl	ethazol		
chloroneb			
BAS 3201F			
carboxin			
thiram			
BAS 3191F			

* the concentration required to inhibit radial growth by 50%.

Table V Ranking of fungicides according to the lowest concentration tested which completely inhibited mycelial growth.

<u>Lowest concentration tested which completely inhibited mycelial growth</u> (ug/ml a.i.)					
< 5	5-10	50	50-100	100	> 100
MEMC	chloroneb	fuberidazole	mancozeb	ethazol	carboxin
thiabendazole	thiram	BAS 3192F	metiram	captan	oxycarboxin
BAS 3201F					PCNB
benomyl					

Figure 10 Effect of various rates of fungicide, incorporated in PDA, on three isolates of R. solani expressed as percentage inhibition of radial growth.

- A. carboxin
- B. oxycarboxin
- C. fuberidazole
- D. BAS 3192F
- E. mancozeb
- F. metiram
- G. captan
- H. PCNB
- I. ethazol
- J. benomyl
- K. thiabendazole
- L. BAS 3201F
- M. thiram
- N. chloroneb

FIGURE 10 A CARBOXIN

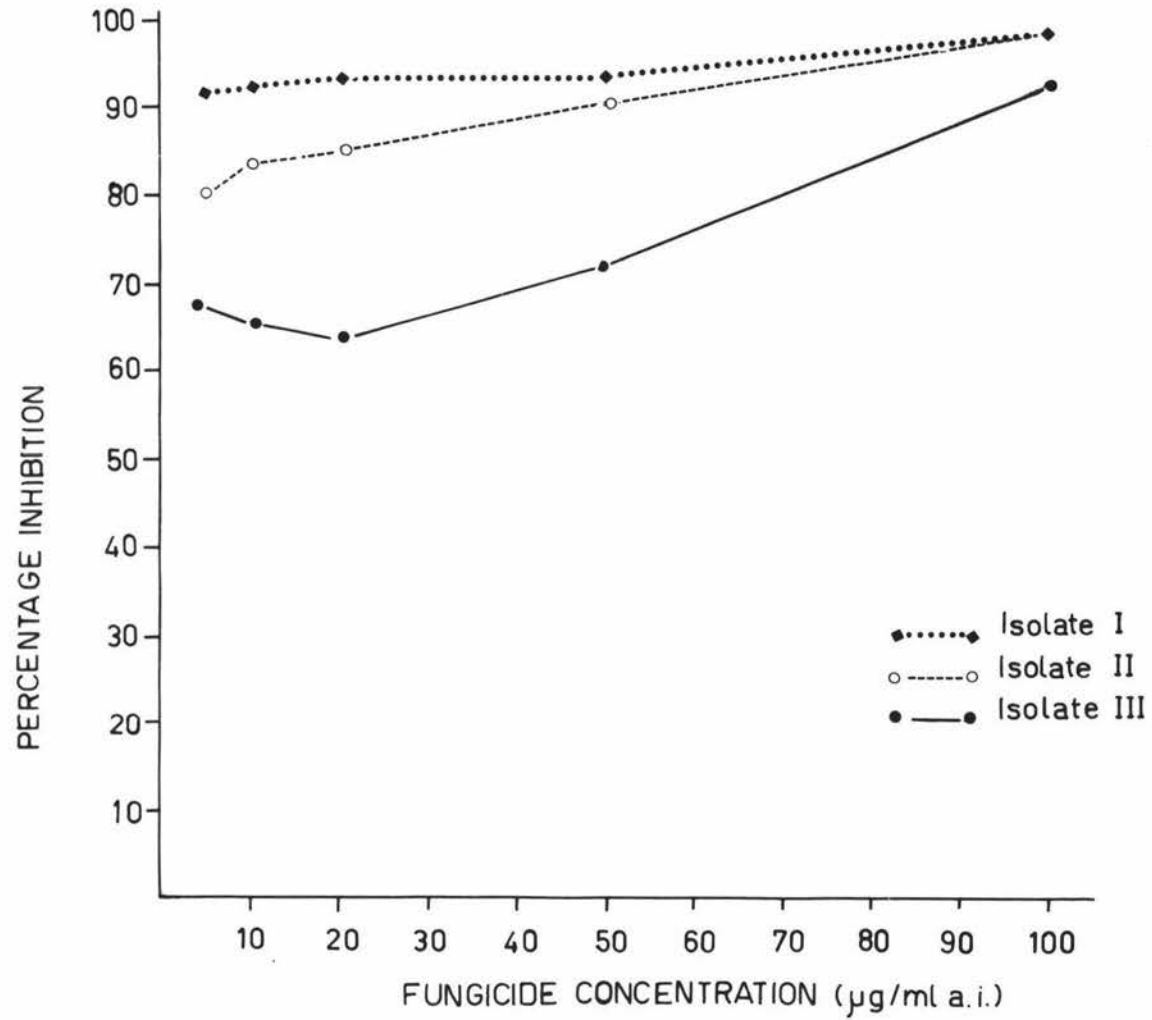


FIGURE 10 B OXYCARBOXIN

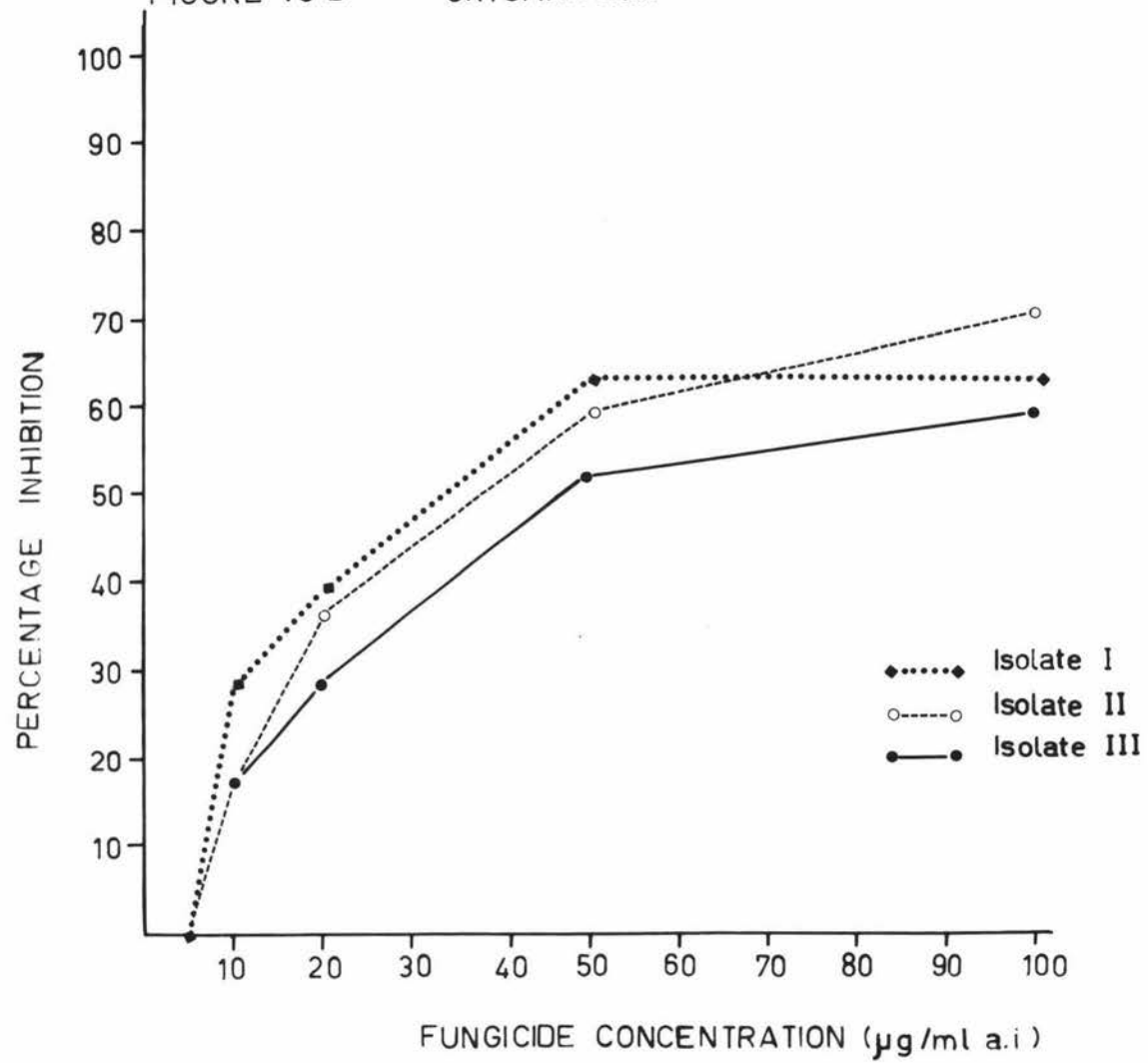


FIGURE 10 C FUBERIDAZOLE

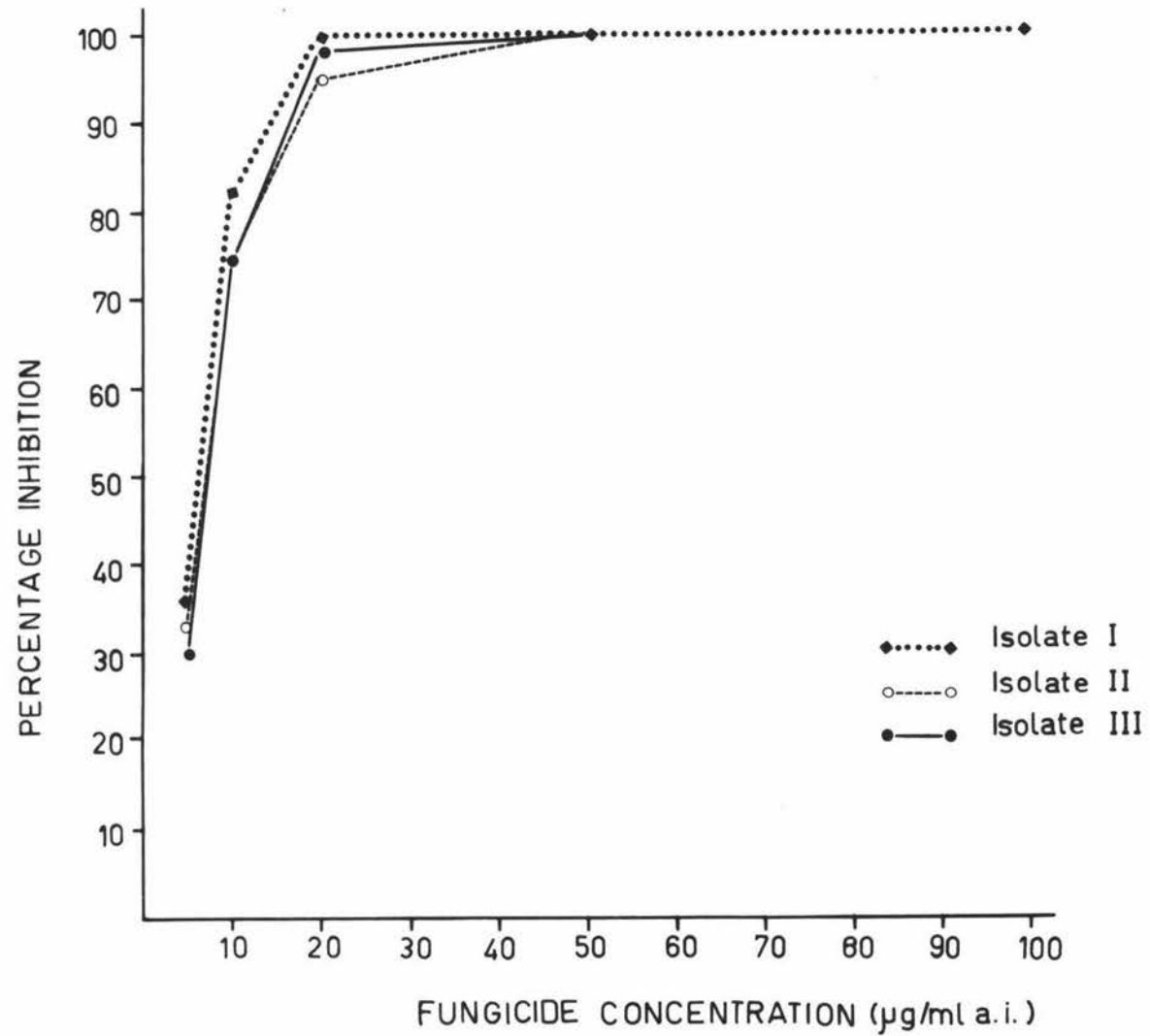


FIGURE 10D BAS 3192F

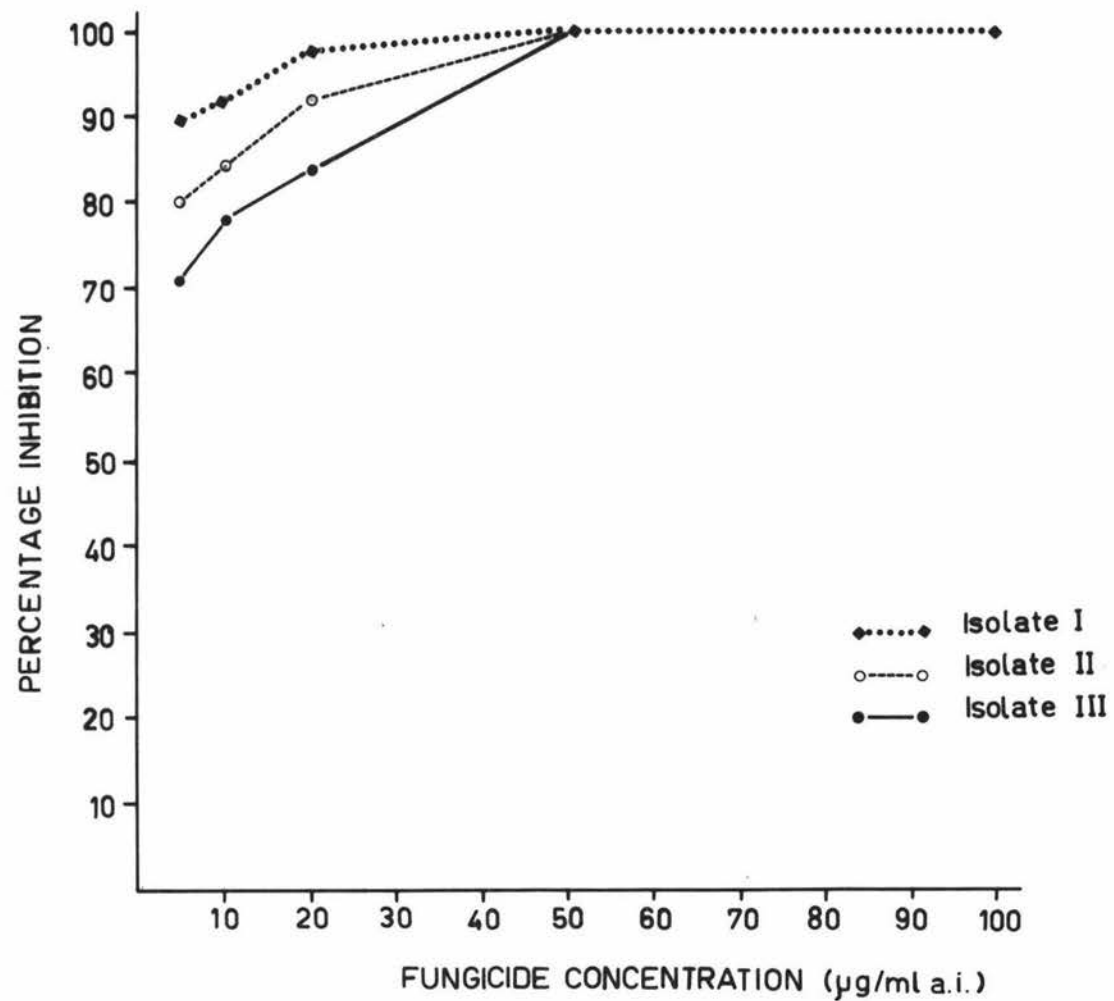


FIGURE 10 E MANCOZEB

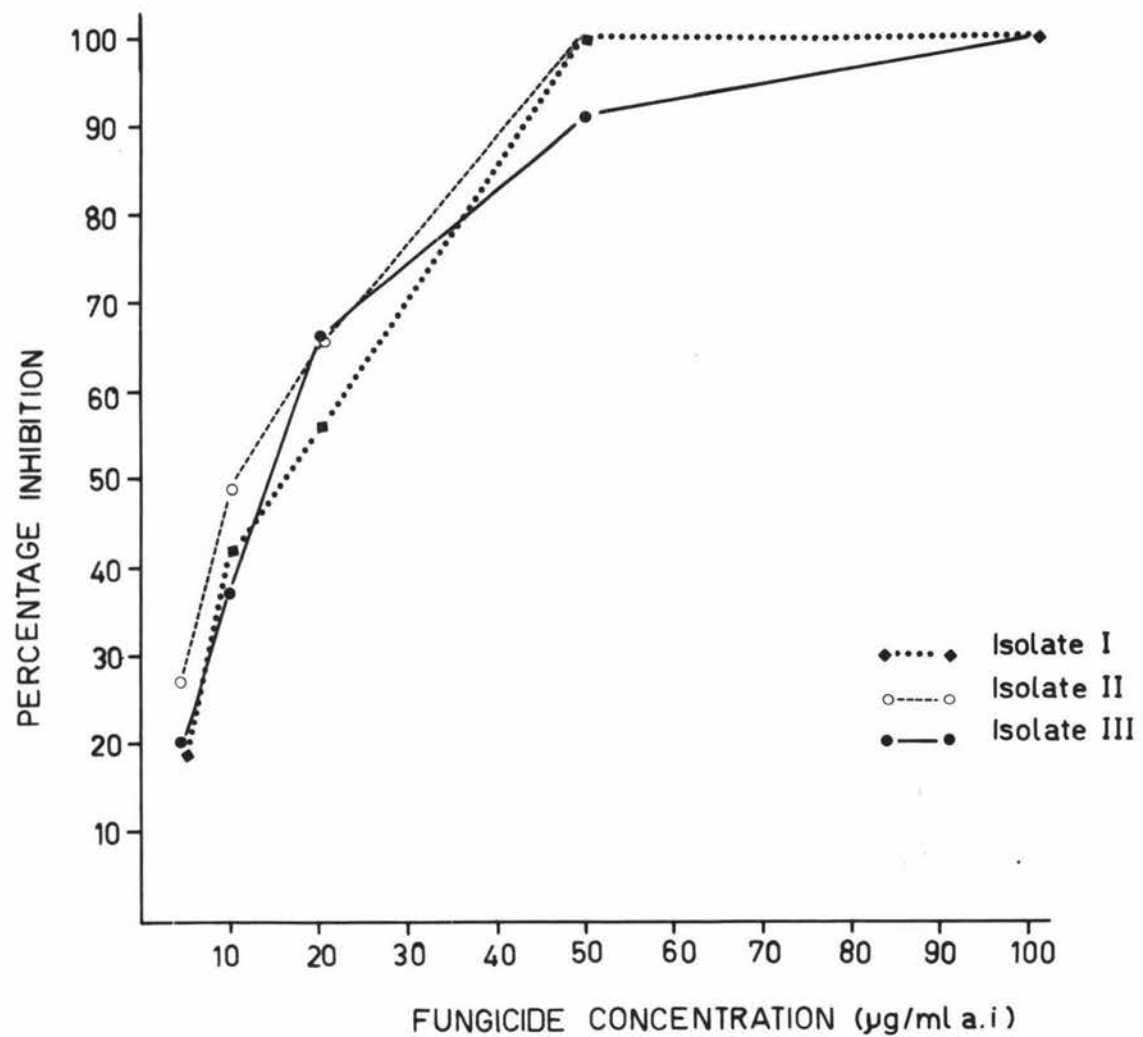


FIGURE 10 F METIRAM

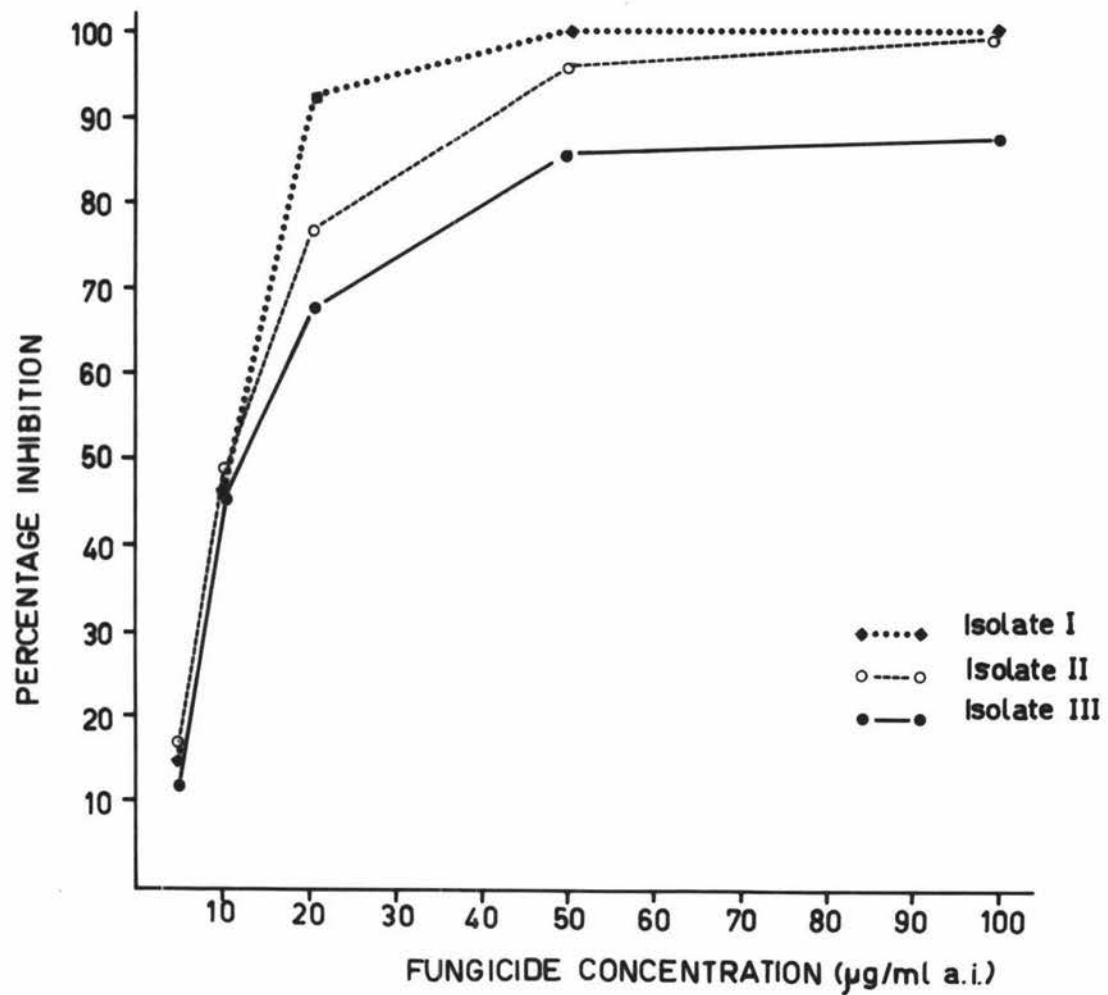


FIGURE 10 G CAPTAN

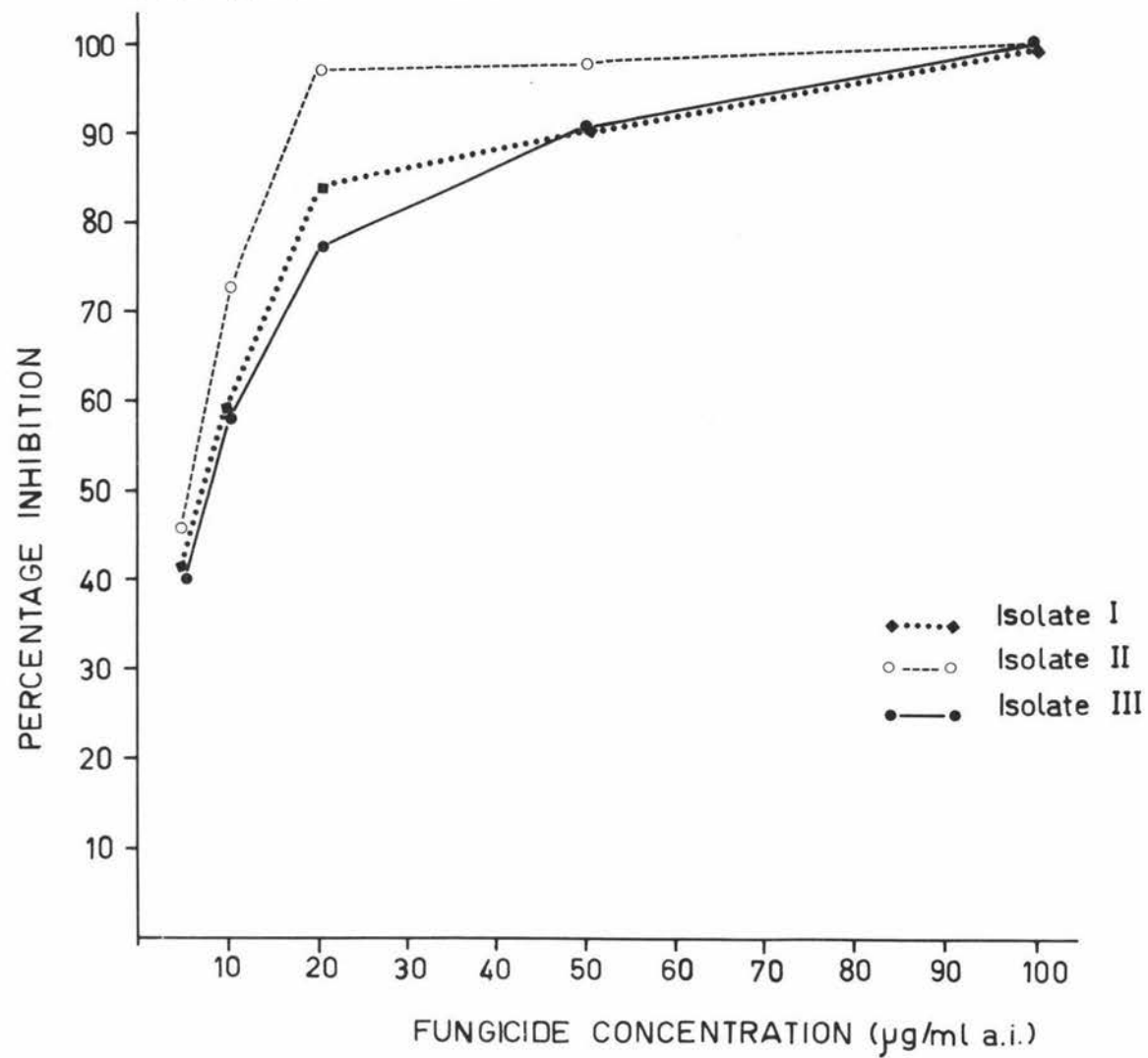


FIGURE 10 H PCNB

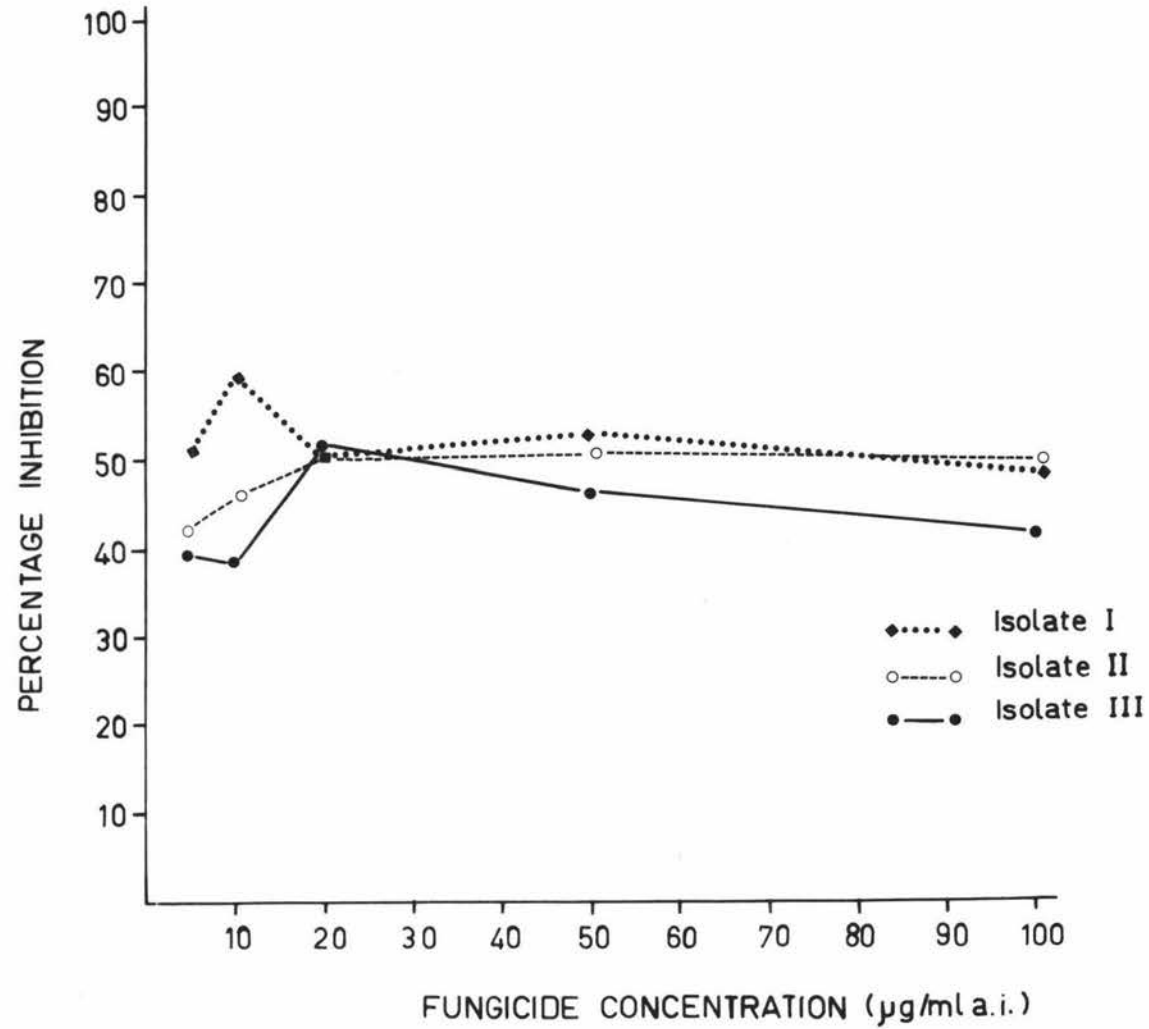


FIGURE 10 I ETHAZOL

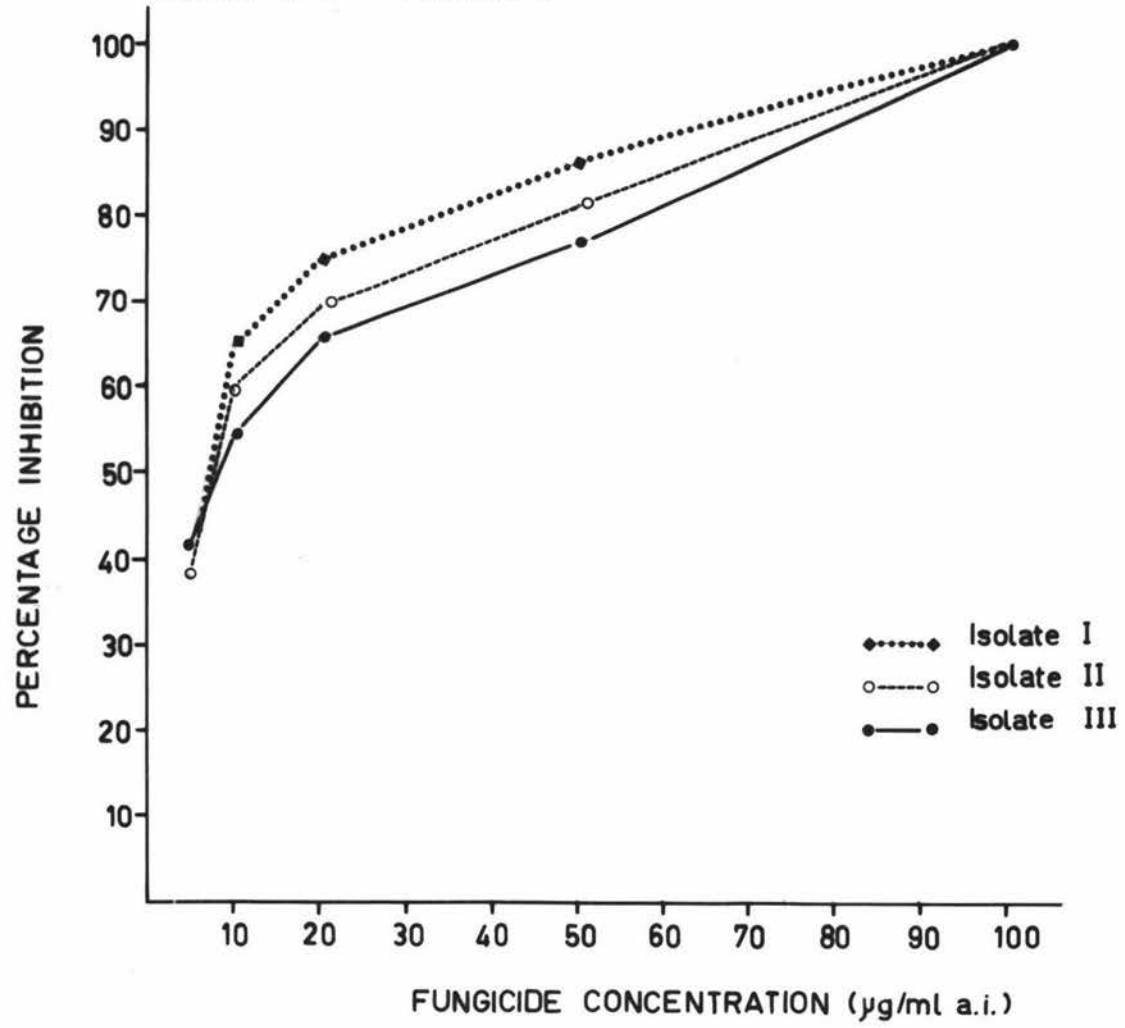


FIGURE 10 J BENOMYL

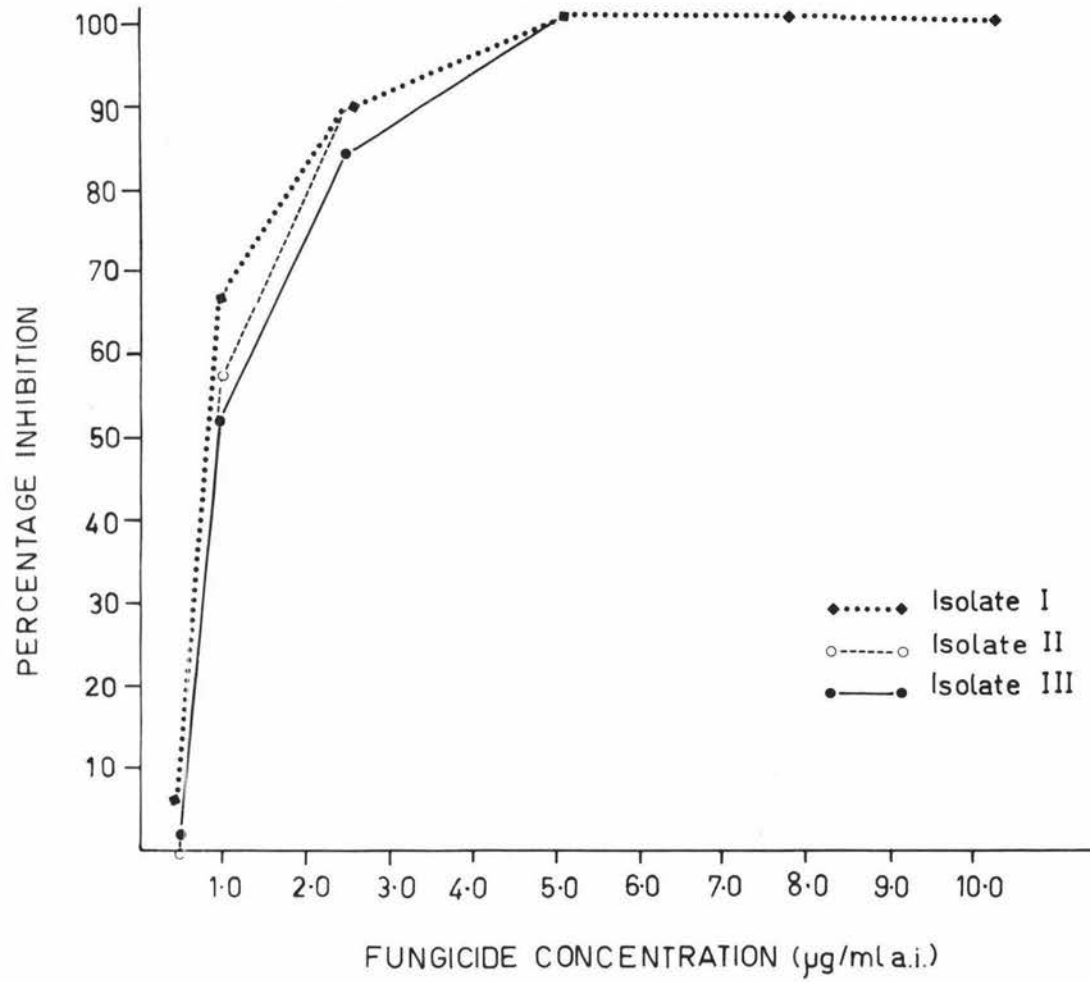


FIGURE 10 K THIABENDAZOLE

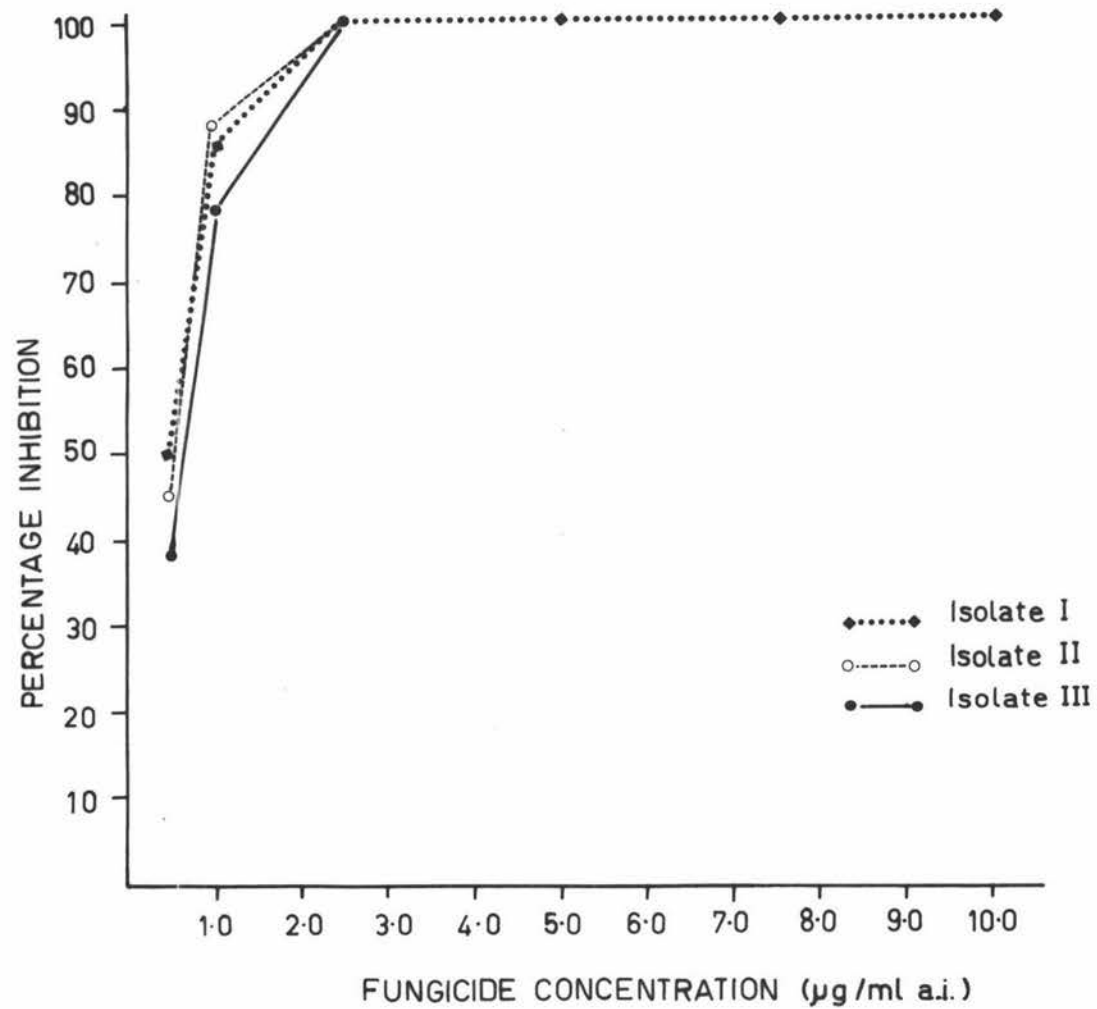


FIGURE 10 L BAS 3201F

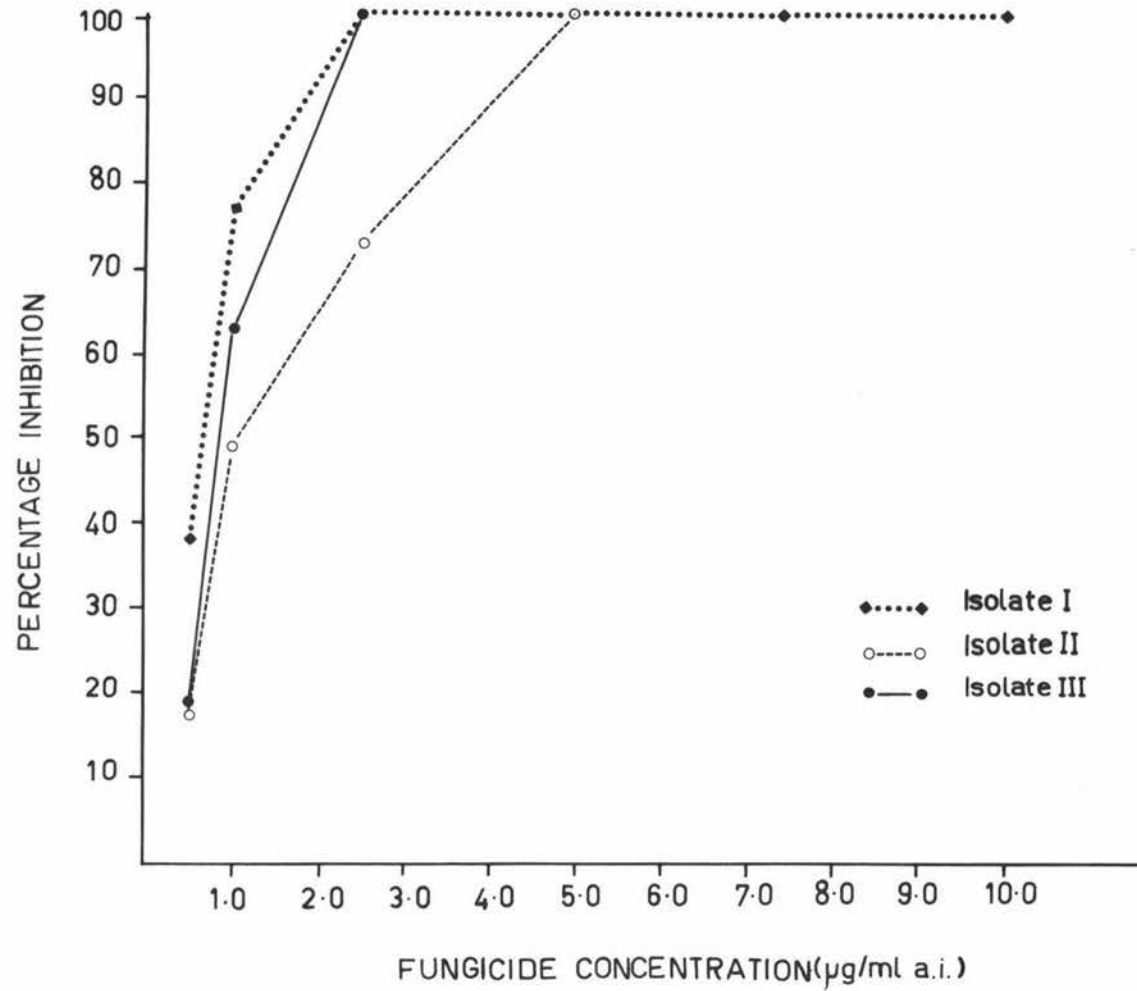


FIGURE 10 M THIRAM

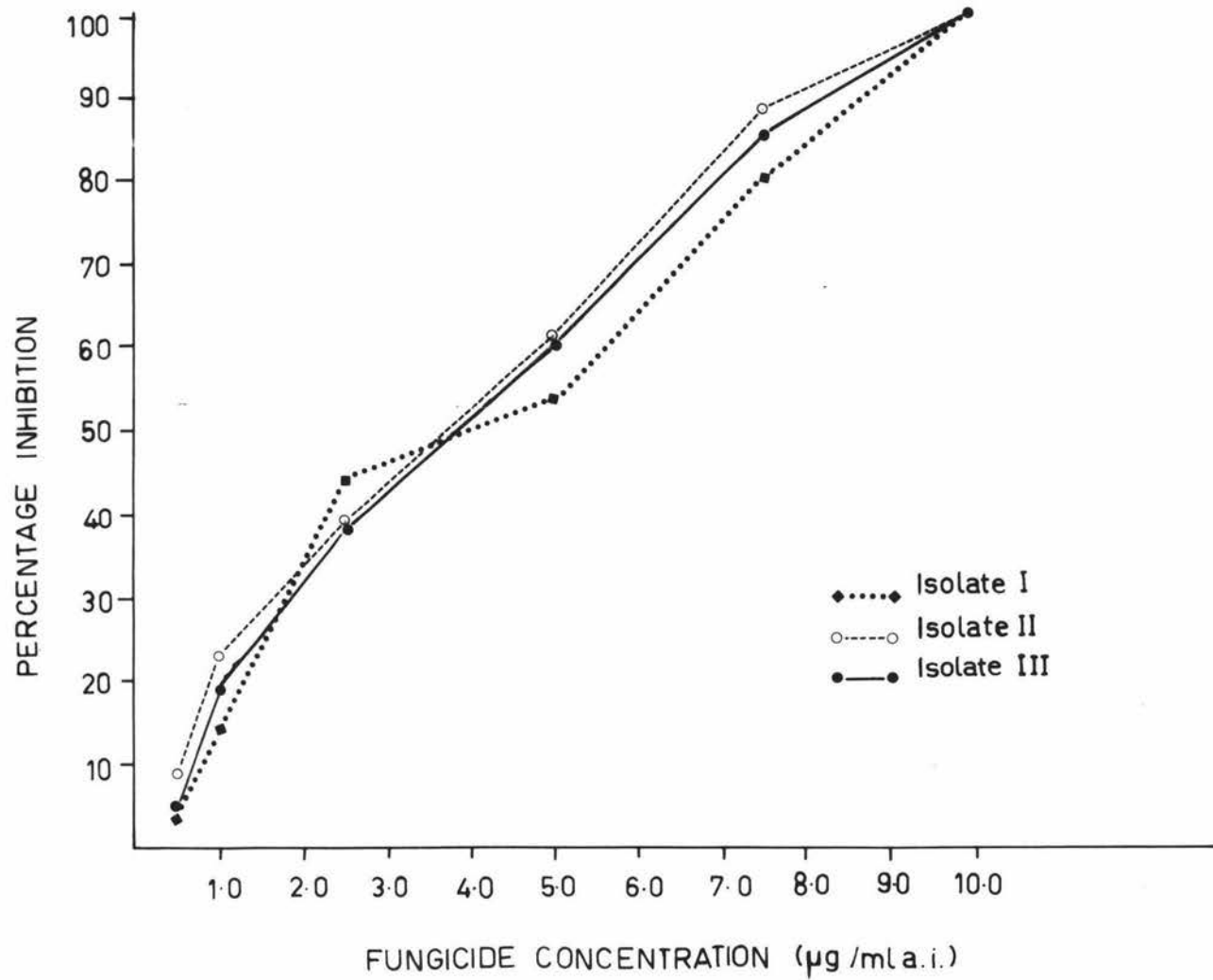
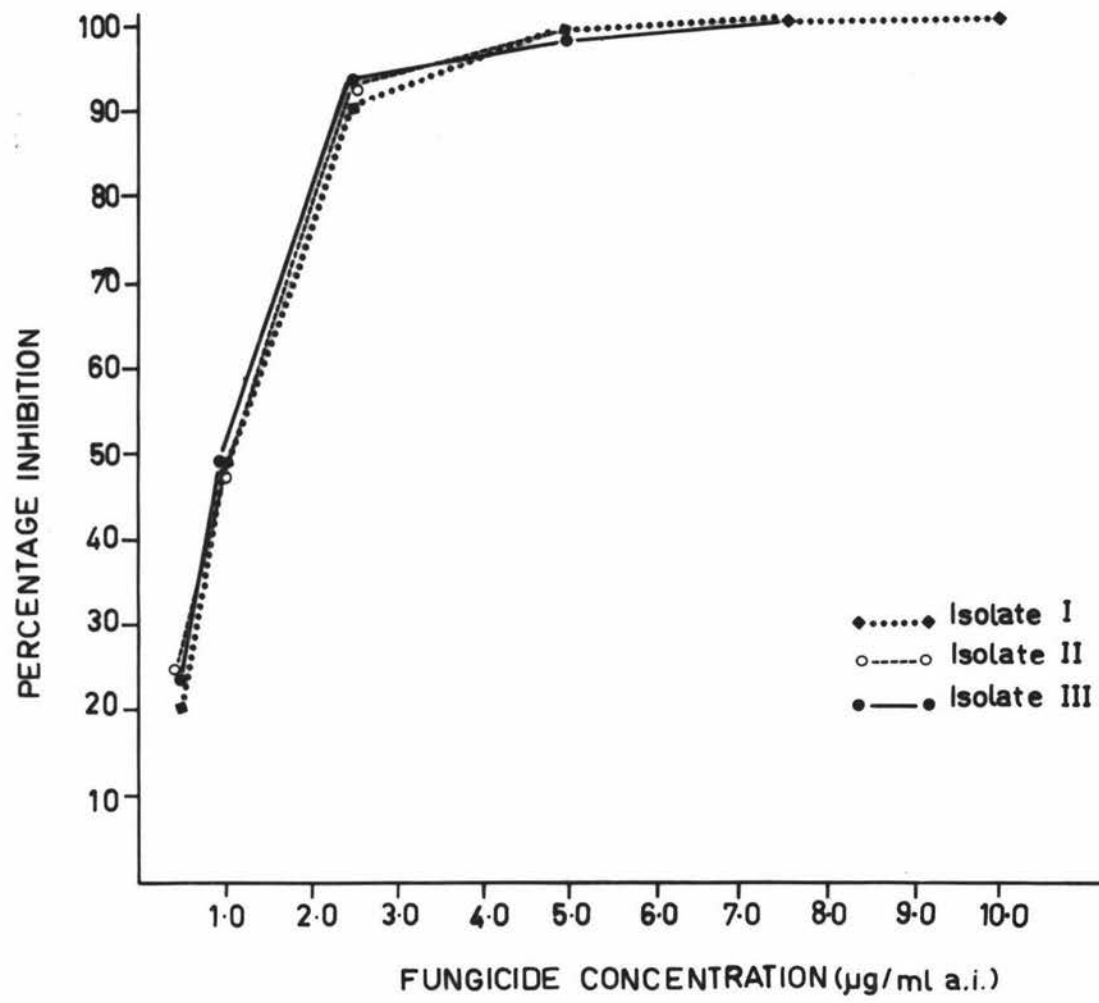


FIGURE 10 N CHLORONE B



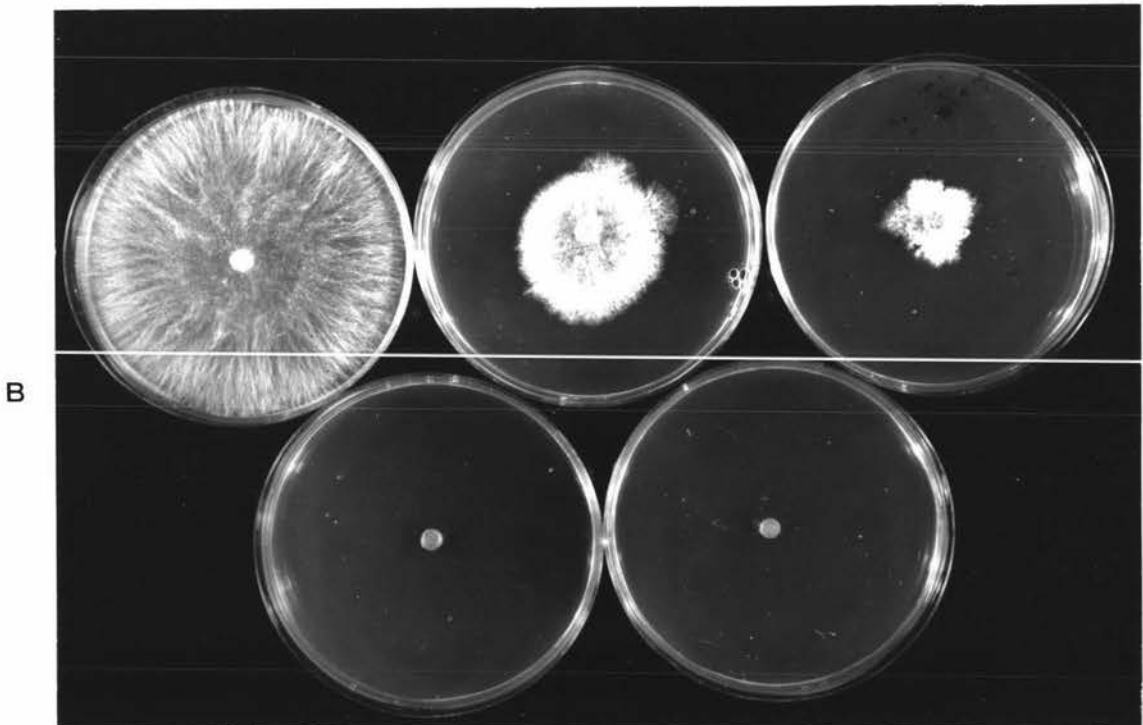
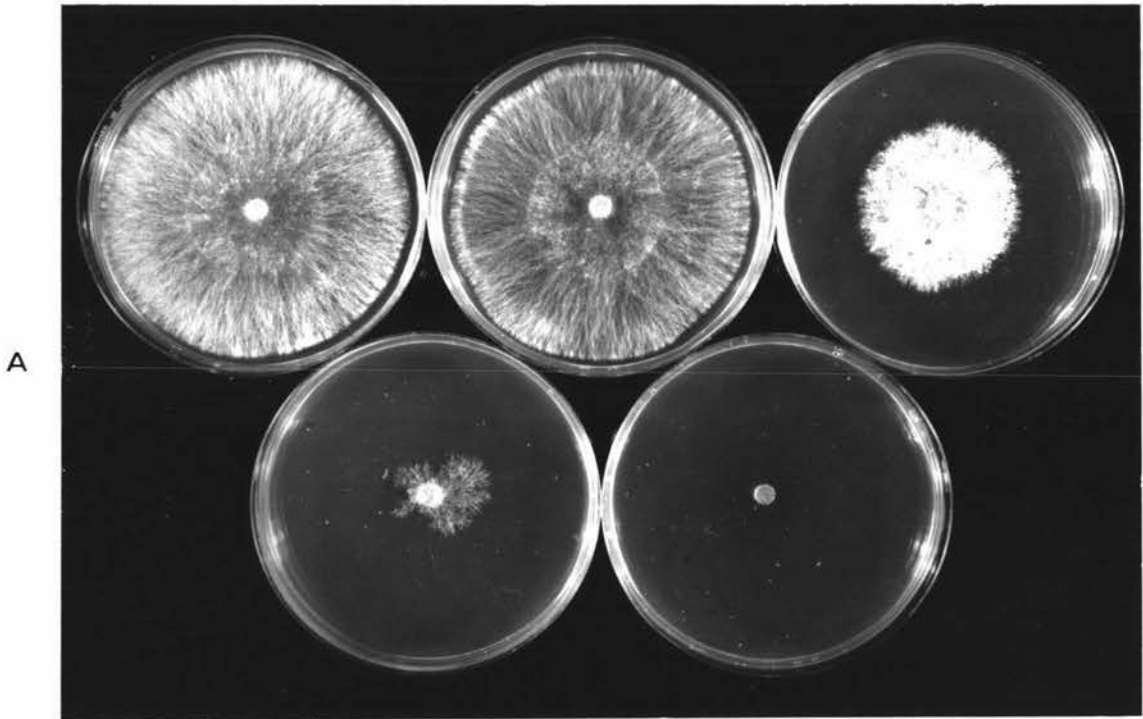


Figure 11 Growth of *R. solani* on PDA₁ plates containing fungicide at various rates. Top row, left to right, plates contain 0, 0.5, 1.0 ug/ml a.i. respectively. Bottom row, left 2.5 ug/ml a.i., right 5.0 ug/ml a.i.

A. benomyl
B. thiabendazole

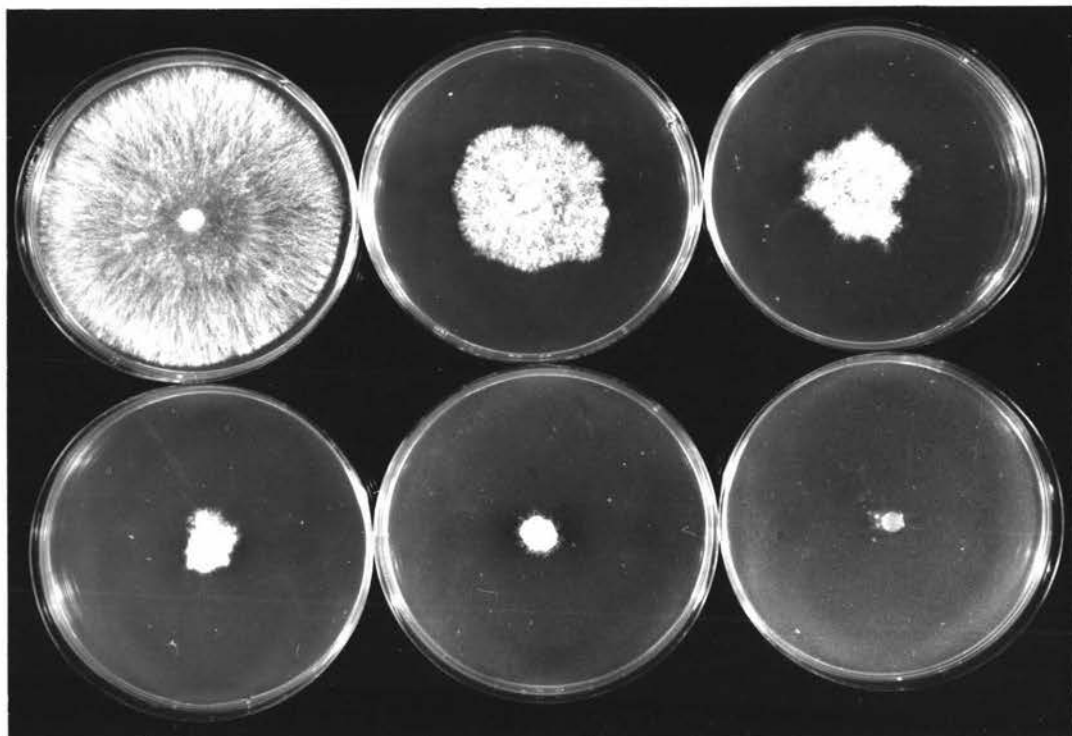


Figure 12 Growth of *R. solani* on PDA_L plates containing captan at various rates.
Top row, left to right, plates contain 0, 5, 10 µg/ml a.i. respectively.
Bottom row, left to right, plates contain 20, 50, 100 µg/ml a.i. respectively.

All fungicides tested showed some activity against R. solani, but some were obviously more effective than others. The organic mercury compound (MEMC) was the most effective, there being complete suppression of growth at the lowest concentration tested (0.5 ug/ml a.i.), and PCNB and oxycarboxin the least effective. In addition to MEMC, seven other fungicides were highly toxic to R. solani with ED₅₀ values of less than 5 ug/ml a.i.

When the fungicides are rated as in Table V, the most notable difference from the ED₅₀ rankings is the relegation of carboxin. It should be noted that results in this experiment were obtained from radial growth measurements only, no account being taken of the density of mycelial growth. Carboxin, even at low concentrations, markedly reduced the density of growth of R. solani as compared with controls and other chemicals. Thus radial growth measurements may not have given a true assessment of the effectiveness of this fungicide.

The results obtained for the three isolates of R. solani were similar. However, some differences did occur, particularly when considering the lowest concentration of fungicide which completely inhibited mycelial growth, (e.g. BAS 3201F, metiram and mancozeb). Isolate III, which was the slowest growing, appeared to be more resistant to some fungicides than the other two isolates. Differences in the sensitivity of different isolates of R. solani to various fungicides has been noted by Sinclair (1960), Maier (1962), Thomas (1962) and Shatla and Sinclair (1963).

When mycelial plugs showing no growth were transferred to fresh PDA_L the effect of all fungicides tested, except MEMC at 2.5 ug/ml a.i. and above, thiram at all concentrations tested, and mancozeb at 100 ug/ml a.i., was shown to be fungistatic. The results obtained with MEMC and mancozeb demonstrate that both fungistatic and fungicidal activities can be produced by the same chemical, depending on its concentration. This effect was also noted by Neely and Himelick (1966). The time of exposure of a fungus to a particular chemical is also known to influence fungi-

static/fungicidal properties (Lilly and Barnett, 1951).

A summary of the results obtained by other investigators using the poison food technique with R. solani is presented in Table VI. The results obtained in the present study are essentially similar to those in Table VI, but with some exceptions. These differences can probably be attributed to variation in sensitivity of the different isolates used.

4. Conclusion: The problem of interpreting these results is a major one. It is possible to rank the fungicides according to their relative abilities in inhibiting mycelial growth but where does one draw the line between effective and non-effective fungicides in terms of likely field performance? Even the least effective fungicides in the above tests may provide adequate control of the disease in a field situation if sufficient fungicide is used. However, taking into account such factors as economics, phytotoxicity and residues, the most acceptable fungicide for field use will be the one active at the lowest concentration, all other factors being equal.

Benomyl and MEMC were selected for further laboratory studies and together with thiabendazole, carboxin, mancozeb, captan and metiram for glasshouse and field trials. MEMC was included for comparative purposes and the other six are either currently recommended for seed-tuber treatment in New Zealand, or are in experimental use. A further consideration in selecting the fungicides was their varied ability to inhibit mycelial growth of R. solani in the foregoing poison food experiments.

Table VI Results of poison food assays obtained with R. solani by other investigators.

<u>Fungicide</u>	<u>ED₅₀</u> (ug/ml a.i.)	<u>Concentration</u> <u>at which com-</u> <u>plete inhibi-</u> <u>tion obtained</u> (ug/ml a.i.)	<u>Investigators</u>
carboxin	< 8.0		Edgington, Walton and Miller (1966)
	< 1.0+		Edgington and Barron (1967)
		5.0*	Borum and Sinclair (1968)
	< 0.5	> 50	Mathre (1968)
	0.1+		Snel, <u>et al.</u> (1970)
oxycarboxin	approx.	10.0 (90% inhib.)	Ragsdale and Sisler (1970)
	80.0	sign. inhib. at 5.0	Davis and Pinckard (1971)
	20-50 3.6		Edgington <u>et al.</u> (1966) Mathre (1968) Snel <u>et al.</u> (1970)
benomyl		2.5	Al-Beldawi and Pinckard (1970)
	< 2.5		Bollen and Fuchs (1970)
		sign. inhib. at 5.0	Davis and Pinckard (1971)
	3.0+		Edgington, <u>et al.</u> (1971)
thiram	< 3.0		Bollen (1972)
		15 100*	Vaartaja (1960) Kendrick and Middleton (1954)
captan		10* 100	Vaartaja (1960) Kendrick and Middleton (1954)
	chloroneb		5-8
< 15		150*	Darrag and Sinclair (1969)
		sign. inhib. at 0.25	Davis and Pinckard (1971)
ethazol		30**	Al-Beldawi and Sinclair (1969)
		sign. inhib. at 0.5	Davis and Pinckard (1971)

ctd. over

Table VI (ctd)

Fungicide	ED ₅₀ (ug/ml a.i.)	<u>Concentration at which com- plete inhibi- tion obtained</u> (ug/ml a.i.)	<u>Investigators</u>
PCNB	0.75+	10* No inhib. at 350*	Kendrick and Middle- ton (1954) Vaartaja (1960) Eckert (1962)

* Large differences from results in Table V.

** Complete inhibition at 30 ug/ml after 76 hours but sparse growth after this time.

+ Converted from molar concentrations.

B. THE EFFECT OF BENOMYL AND MEMC ON THE VIABILITY OF SCLEROTIA

1. Introduction: Assessment on agar of the viability of sclerotia removed from tubers treated with fungicides is a technique widely used in studies relating to black scurf control (Melhus and Gilman, 1921; Thurston, 1921; Cunningham, 1925a, 1925b; Schaal, 1939; Chamberlain, 1932a; Sanford and Marritt, 1933; Graham, Srivastava and Foister, 1957; Graham, 1960; Kooistra, 1971).

In the following experiments this method was used to determine:

1. The effect of dipping time on the viability of sclerotia from seed tubers immersed in varying concentrations of benomyl and MEMC.
2. The efficiency of the mercury (MEMC) dip treatment of seed tubers as practised by farmers.

2. Materials and Methods:

Experiment I: Suspensions of benomyl and solutions of MEMC of the following concentrations were prepared in 500 ml of tap water:

benomyl	-	0, 1000, 2500, 5000, 7500	ug/ml a.i.
MEMC	-	0, 50, 100, 150, 200, 300	ug/ml a.i.

Five tubers with abundant sclerotia were dipped for 1, 5, 15 and 30 minutes in each concentration of benomyl and for 0.5, 1, 5 and 15 minutes in each concentration of MEMC. On removal the tubers were allowed to dry and 50 to 60 sclerotia (avoiding the very thick and very thin) from each treatment were placed (five per plate) on water agar containing 50 ug/ml each of streptomycin and penicillin. After two days incubation in the dark at 24C germinated sclerotia were removed from each plate to prevent developing mycelium from overgrowing non-germinated sclerotia. Final assessment of viability was made after a further two days incubation. With practice it was possible to macroscopically recognise the characteristic hyphae of R. solani emanating from sclerotia (Figure 13). However, depending on the particular

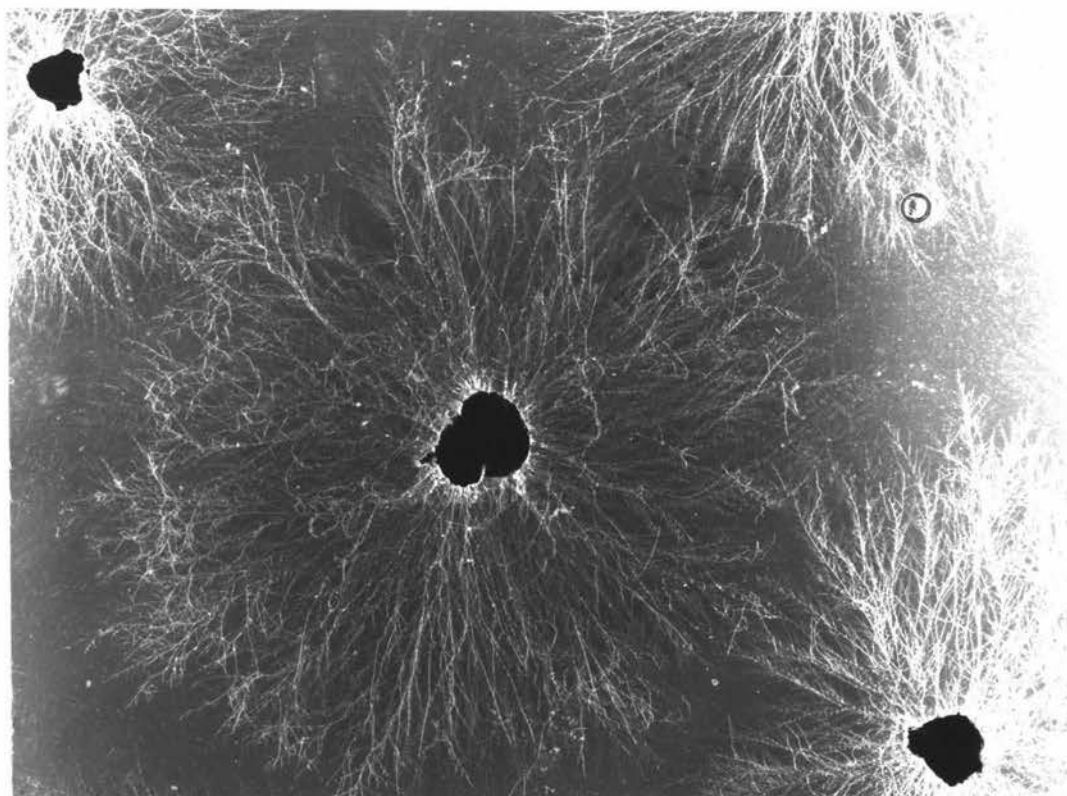
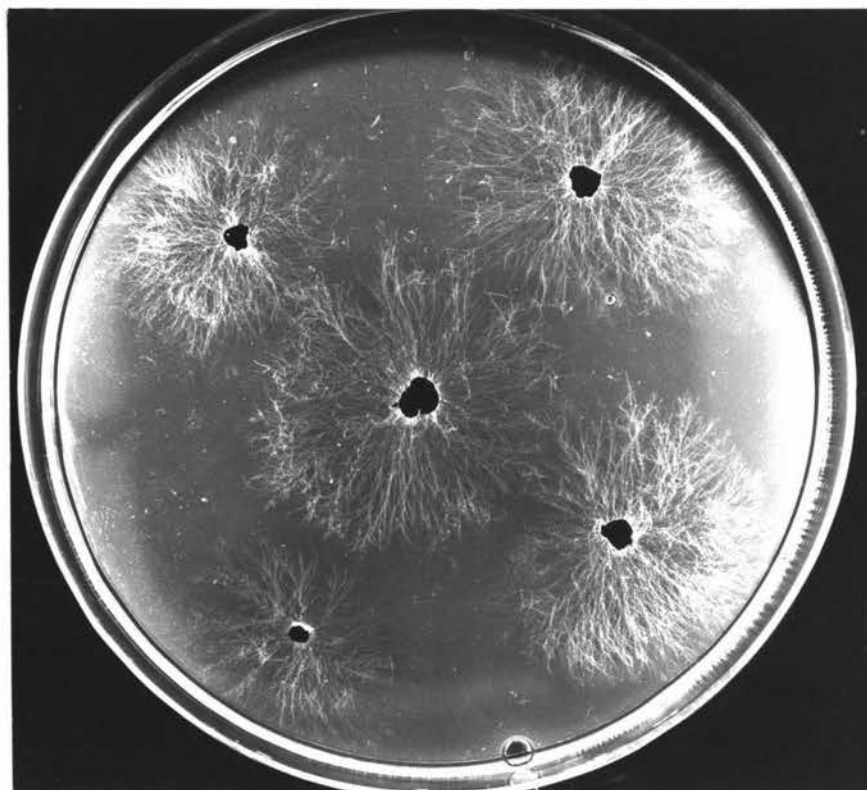


Figure 13 Germination of sclerotia of R. solani on WA after three days incubation at 24C.

fungicide and concentration used, fungal and bacterial contaminants often grew out from sclerotia, in which cases it was necessary to confirm identification by examination under a compound microscope (x 100).

Experiment II: Sample tubers were obtained of four seed lines dipped in MEMC by commercial potato growers. The viability of 50 to 100 sclerotia from ten tubers of each seed line was determined following the method described above.

3. Results and Discussion:

Experiment I

(a) benomyl. The results (Table VII) show that at all concentrations increased dipping time failed to consistently influence the germination capacity of sclerotia. This is understandable since benomyl acts fungistatically (Kooistra, 1971). That is, in this experiment dipping provided a coating of fungicide only to the exposed surface of sclerotia, which meant that on agar, mycelium was able to develop from that surface which had been in contact with the tuber. This also explains the inability of benomyl to prevent the development of mycelium from sclerotia at concentrations very much greater than those which completely inhibited mycelial growth in the poison food tests, (Chapter II, A).

Table VII Effect of dipping time and concentration of benomyl on viability of sclerotia.

Conc. ug/ml a.i.	Time of dipping (minutes)			
	1	5	15	30
	% germination of sclerotia			
0	100	90	91	90
1000	63	86	76	73
2500	23	19	41	20
5000	2	10	14	0
7500	15	0	3.5	8

The results also show that for each treatment time an increase in concentration of benomyl was associated with decreased percentage germination of sclerotia. This raises the question of whether some fungicidal activity may occur at the higher concentrations and in turn whether, when used as a dip treatment, benomyl could serve as a replacement for the organic mercury fungicides. However, to be acceptable there would need to be a greater degree of effectiveness in inactivating sclerotia. This could possibly be achieved by the addition of adjuvants such as wetting agents and mineral oils, or by solubilization of these fungicides with acids. Graham (1960) demonstrated that the addition of wetting agents to mercury fungicides markedly increased their effectiveness in killing sclerotia. Further, when mineral oils were added to benomyl, thiabendazole and carboxin more effective control of vascular and foliage diseases was obtained by Solel, Pinkas and Loebenstein (1972) and Smith and Crosby (1972) which they suggested may be due to enhanced penetration of plant tissues by the fungicides. Buchenauer and Erwin (1971, 1972) obtained control of Verticillium wilt of cotton by spraying with acid solutions of benomyl and thiabendazole whereas non-acidified suspensions had no effect on the disease.

(b) MEMC. The results (Table VIII) show that at concentrations of 50, 100 and 150 ug/ml a.i. an increase in

Table VIII Effect of dipping time and concentration of MEMC on viability of sclerotia.

Conc. ug/ml a.i.	Time of dipping (minutes)			
	0.5	1	5	15
	% germination of sclerotia			
0	96	96	94	96
50	50	44	24	0
100	14	10	0	0
150	8	2	0	0
200	0	0	0	0
300	0	0	0	0

dipping time from 0.5 to 15 minutes provided a progressive improvement in the lethal effect of MEMC on sclerotia. This is explained by the fact that the organic mercuries are fungicidal in action (Kooistra, 1971), which requires that dipping be of sufficient duration to enable complete penetration of sclerotia.

The results also show that for each treatment time the effectiveness of MEMC increased as the concentration increased. It should be noted that the figures in Table VIII record only the percentage of sclerotia germinated and take no account of the vigour of germination. Even at 50 ug/ml a.i. and dipping times of 0.5 and 1 minute those sclerotia which germinated did so only slowly and the mycelium which developed was relatively sparse. This applied more particularly at the higher concentrations. Taking this into account it would seem that the commercial recommendation of 0.5 to 1 minute dip in a solution containing 150 ug/ml a.i. (0.5 lb Baytan Forte/20 gal water) provides an effective means of controlling tuber-borne inoculum.

Experiment II: The results (Table IX) show that up to 69% of sclerotia from seed tubers dip treated by farmers were viable. Several factors could account for this apparent ineffectiveness of the treatment in field practice.

Table IX Efficiency of MEMC dipping as practised by commercial potato growers.

	Test			
	1	2	3	4
	% germination of sclerotia			
Dipped	8	7	69	22
Non-dipped	91	82	-	-

1. Under commercial conditions approximately one ton of seed is required to plant an acre, and depending upon the acreage involved so regular fortification of the dipping solution and the preparation of fresh solutions may be necessary. Failure to follow the manufacturer's specific recommendations in this regard could result in reduction of the solution concentration to a level rendering it ineffective.
2. Depending on ground conditions at the time of lifting and subsequent handling of tubers so large amounts of soil can adhere to seed tubers. The protection thus afforded sclerotia could prevent sufficient fungicide penetrating to be effective.
3. The length of time of dipping is probably not a critical factor since it would be difficult to have tubers in contact with the fungicide for less than one minute and tubers, after dipping, often remain wet for long periods of time. For example, in one commercial system described by Wilson (1971) tubers remained wet for up to six weeks. However, where tubers are allowed to dry rapidly and one or both of the above factors operate also, length of dipping time may be of significance.

Chapter III

GLASSHOUSE AND FIELD TRIALS

The poison food tests (Chapter II) revealed several fungicides to be highly toxic to *R. solani* at low concentrations. However, their performance in control of the black scurf disease was not known. Accordingly, a series of glasshouse and field studies were conducted aimed specifically at determining the relative effectiveness of the chemicals in preventing shoot infections arising from tuber-borne inoculum. Except for MEMC the fungicides (benomyl, thiabendazole, carboxin, metiram, captan, mancozeb) were applied as dusts to seed tubers, as recommended by the manufacturers.

A. GLASSHOUSE TRIALS

1. Materials and Methods: The tubers used were from a certified seed line of the variety Ilam Hardy, and carried abundant sclerotia. Groups of 60 tubers were selected at random and the following treatments applied:

benomyl	}	10% a.i. dusts	}	Applied at rate of 10g dust/kg tubers
thiabendazole				
carboxin				
metiram				
captan				
mancozeb		8% a.i. dust	}	Applied at rate of 10g dust/kg tubers
MEMC		1 min. dip in a solution containing 150 ug/ml Hg.		

Each dust treatment was applied by agitating the appropriate amount of fungicide with the 60 tubers in a plastic bag. On removal of the tubers the plastic bags were weighed to ascertain how much dust had actually adhered to the tubers.

In the organo-mercury dip treatment 60 tubers were immersed in MEMC for one minute.

Controls were provided by 60 untreated tubers.

Treatments were applied eight days before planting in field soil (Manawatu silt loam, pH 6.5). Since the soil was from a field in which potatoes had been grown four years previously there was a possibility of the results reflecting control of both tuber-borne and soil-borne inoculum. This problem was overcome by using heat treated soil (80-90C for one hour) as a control. That is, for each tuber treatment 30 tubers were planted in treated soil, and the remainder in non-treated soil.

Tubers were planted in the soil at a depth of 10 to 13 cm in black polythene bags* (Figure 14) which were randomly set out in a heated glasshouse, the air temperature of which ranged between 13C and 35C.

After 22 days when plants were 7 to 10 cm high (Figure 14), tubers were dug and the shoots assessed for severity of disease using a visual rating, as follows:

- 0 - no lesions
- 1 - trace superficial lesion
- 2 - intermediate stage
- 3 - shoot severely lesioned or girdled but not dead
- 4 - shoot dead

From these figures a disease index was calculated for each treatment, following the formula devised by Davis. et al. (1971), namely,

$$\text{Index} = \frac{\sum (\text{class}^2 \times \text{number of shoots in a given class})}{\text{total number of shoots per treatment}}$$

For each treatment the number of emerged shoots was also recorded as a percentage of the total shoots produced.

At the completion of the foregoing experiment the effectiveness of the different tuber treatments in inactivating sclerotia was investigated. Twelve tubers from each treatment were washed thoroughly to remove the associated fungicide, allowed to dry and the viability of sclerotia

* These bags are manufactured by Plastic Products Ltd., Hamilton, and in the horticultural trade are known as 'planter bags', which term is used hereinafter.



Figure 14 Planter bags used in glass-house trials. Potatoes after three weeks growth in non-treated soil.

(50 per treatment) was assessed by plating to water agar, following the method previously described (p. 49).

2. Results and Discussion:

(i) Shoot infections

The results are summarized in Table X and Figure 15.

- (a) Non-treated Soil: The fact that 43% of shoots from the untreated (control) tubers were killed indicated a relatively high inoculum load being associated with the seed, and the environmental conditions being conducive for establishment and development of infections.

All treatments gave some measure of control of the disease. However, the mercury dip treatment was outstanding in that total control was achieved. Benomyl, thiabendazole and carboxin were also highly effective, with relatively few shoots exhibiting lesions. Metiram, mancozeb and captan were less effective but still gave appreciable control.

The emergence figures also reflect the degree of disease control achieved. That is, for each treatment there was a strong correlation between the percentage shoot emergence and the disease index.

- (b) Treated Soil: In heat treated soil the disease index of the control series was only slightly greater than in non-treated soil, indicating the soil in this experiment to be an insignificant source of inoculum. Fewer shoots were killed and this is reflected in a higher percentage emergence. However, it should be noted (Table X) that many shoots (treated soil) were in category 3, suggesting that in time some of these would have succumbed.

Of the treatments MEMC, benomyl, thiabendazole and carboxin were most effective, almost total control being provided by each.

Conversely, relatively little control was provided by metiram, captan and mancozeb.

Table X Efficiency of fungicides in controlling shoot infections when applied as tuber treatments: Glasshouse Trials.

	Emergence (% shoots emerged)	Percentage shoots in each class					Disease Index*
		0	1	2	3	4	
<u>Natural soil</u>							
control	35	29	0	4	24	43	9.20
metiram	75	56	4	9	19	12	4.03
mancozeb	76	60	2	6	24	8	3.70
captan	78	57	2	11	23	7	3.65
benomyl	87	92	5	2	1	0	0.22
carboxin	97	94	4	1	0	0	0.08
thiabendazole	88	94	5	1	0	0	0.09
MEMC	89	100	0	0	0	0	0.00
<u>Treated soil</u>							
control	65	25	5	5	40	25	7.85
metiram	74	41	1	5	34	19	6.31
mancozeb	63	42	8	2	20	28	6.44
captan	60	28	5	5	34	28	7.79
benomyl	86	72	17	1	6	4	1.39
carboxin	89	91	8	1	0	0	0.12
thiabendazole	91	95	4	1	0	0	0.08
MEMC	98	90	8	2	0	0	0.16

$$* \text{ Index} = \frac{\sum (\text{class}^2 \times \text{no. of shoots in given class})}{\text{total no. of shoots per treatment}}$$

Figure 15 Potato shoots from seed tubers treated with various fungicides and planted in non-treated soil.

- A. control (seed tubers non-treated)
- B. benomyl
- C. thiabendazole
- D. carboxin
- E. MEMC
- F. captan
- G. mancozeb
- H. metiram



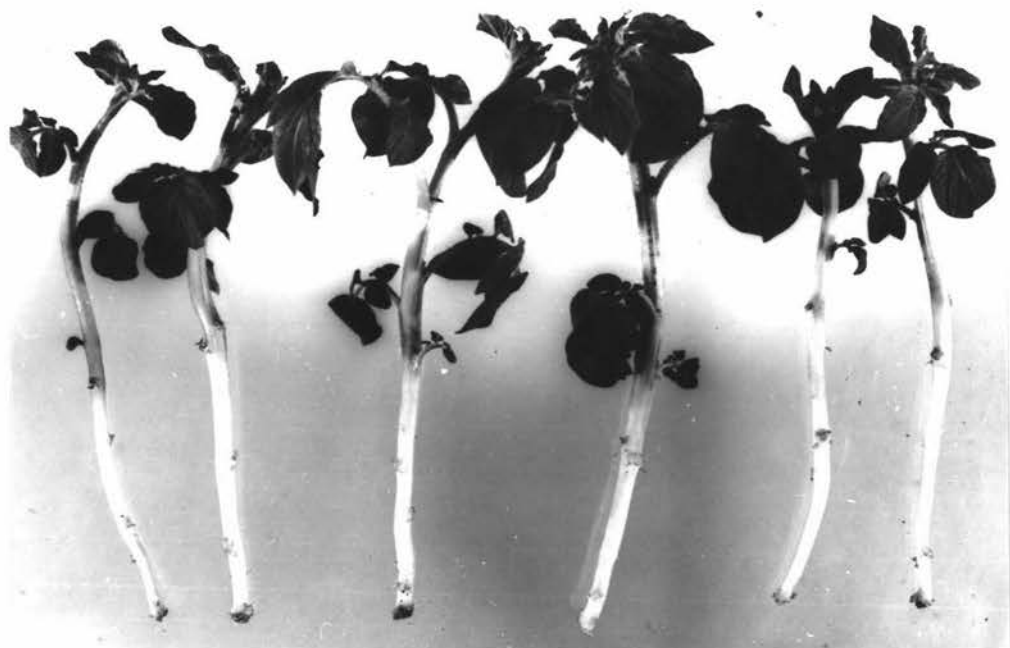
A. control (seed tubers non-treated)



B. benomyl



C. thiabendazole



D. carboxin



E. MEMC



F. captan



G. mancozeb



H. metiram

It is difficult to reconcile the degree of control achieved by metiram, captan and mancozeb in treated soil with that obtained in non-treated soil. An obvious interpretation is that these three fungicides were in fact ineffective against R. solani and that the measure of control obtained in the non-treated soil was simply an expression of antagonism by components of the soil microbial population against tuber-borne inoculum. However, such a hypothesis is invalidated by the fact that the disease index for the control series (tubers not treated) was slightly greater in the non-treated soil than in the treated soil.

Considerable differences were noted in the ability of the fungicide dusts to adhere to tubers. The following are the percentage values of dust adhering to tubers following treatment in plastic bags prior to planting: benomyl, 70%; captan, 50%; mancozeb, 83%; metiram, 63%; thiabendazole, 90%; carboxin, 60%. It is not known whether these differences are due to inherent variation in the ability of the dusts to adhere or are merely the expression of differences in formulation.

It is unlikely that the degree of disease control was influenced by variation in the ability of the dusts to adhere to seed tubers since macroscopically it appeared that total coverage was achieved in all treatments. This raises the question of whether the fungicide application rate (10 g/kg) could be reduced. It should be noted, however, that tuber treatments in these experiments were conducted with small numbers of tubers under ideal circumstances. Because dusting under farm conditions is likely to be much less efficient it would seem prudent to abide by the recommended rate of application.

Since in this study benomyl, thiabendazole and carboxin provided a high degree of disease control it is possible that formulations of each fungicide

containing less than 10% a.i. may be equally effective. This aspect merits further investigation.

It is interesting to note that the fungicides which provided the best control of shoot lesioning under glasshouse conditions (MEMC, benomyl, carboxin, thiabendazole) were those that were most effective in inhibiting mycelial growth of R. solani in poison food tests (Chapter II). It would seem that in this study the poison food test was a satisfactory indication of the likely ability of a chemical to control the black scurf disease. However, many more chemicals would need to be tested to see if this correlation persists before any definite conclusion could be drawn.

(ii) Viability of sclerotia

The results (Table XI) show that, with the exception of MEMC, a large percentage of sclerotia were viable after contact with the various fungicides, indicating that only MEMC was fungicidal. Since benomyl, thiabendazole, carboxin, metiram, captan and mancozeb all reduced the severity of shoot lesions as compared with the control (Table X), one must conclude that these dusts were acting fungistatically. That is, the disease control provided by these chemicals resulted from their presence on the outside of sclerotia preventing the development of mycelium without actually killing sclerotia. In this regard benomyl, thiabendazole and carboxin were obviously more effective fungistats than metiram, captan and mancozeb.

In a field situation it is possible that leaching could remove sufficient dust to allow sclerotia to germinate at some later stage so that, while providing control of shoot lesioning, stolon and root infections could develop. This later germination could also lead to the development of sclerotia on progeny tubers and the establishment of the pathogen in soil which means that these fungistats would not affect the survival of R. solani from one season's crop to the next.

Table XI Viability of sclerotia removed from tubers dusted and planted in soil (for three weeks).

	control	benomyl	thiabendazole	carboxin	metiram	captan	mancozeb	MEMC
Non-treated soil	60*	80	40	28	88	68	72	0
Treated soil	66	82	36	20	66	54	74	2

* figures are % germination of sclerotia

A surprising feature of the results was the relatively low percentage germination of sclerotia from non-treated soil. A possible explanation is that the sclerotia were so depleted by germination during the three weeks in soil as to render them no longer viable.

B. FIELD TRIALS

Four trials using two sclerotia-infected certified seed lines (var. Ilam Hardy) were conducted to determine the relative effectiveness of several fungicides, alone and in combination, in preventing shoot infections under field conditions.

1. Material and Methods

The following treatments were applied in Trials 1, 3 and 4.

benomyl	}	10% a.i. dusts	} Applied at rate of 10 g dust/kg tubers
thiabendazole			
carboxin			
metiram			
captan	}	8% a.i. dust	
mancozeb			
benomyl-captan	}	5% a.i. of each chemical	
thiabendazole-captan			
carboxin-captan			

MEMC 1 min. dip in a solution containing 150 ug/ml Hg.

In Trial 2 only benomyl, carboxin, thiabendazole, captan and benomyl-captan were used.

Untreated tubers served as controls in each trial.

The treatments were applied within the week before planting and for each trial 20 tubers of each treatment were planted 13 to 16 cm deep and 10 to 13 cm apart.

Details of the four Trials are given in the following tables.

TRIAL	SOIL	SEED	DATE PLANTED	TIME UNTIL ASSESSMENT (days)
1	Kairanga silt loam pH 6.3 In potatoes 1971/72	Ilam Hardy line K/1004+	27/9/72	33
2	Manawatu silt loam* pH 6.4 In potatoes 1968/69	Ilam Hardy Line K/1004+	28/9/72	36

* same soil as used in glasshouse trials

+ Same seed line as used in glasshouse trials

TRIAL	SOIL	SEED	DATE PLANTED	TIME UNTIL ASSESSMENT (days)
3	Manawatu silt loam* pH 6.4 In potatoes 1968/69	Ilam Hardy line K/1004+	30/9/72	33
4	Manawatu silt loam* pH 6.4 In potatoes 1968/69	Ilam Hardy line F/1408A	20/10/72	31

* Same soil as used in glasshouse trials

+ Same seed line as used in glasshouse trials

After digging, shoots were assessed for severity of disease, emergence figures were recorded and disease indices calculated, as for the glasshouse trials (p.56).

2. Results and Discussion

The results are summarized in Tables XII and XIII.

With the exception of Trial 1 the results obtained generally reflect what occurred in the glasshouse trials in non-treated soil. That is, benomyl, thiabendazole and carboxin gave excellent control of the disease whereas captan, mancozeb and metiram were less effective but still gave appreciable control. MEMC gave almost total control of shoot lesioning in Trial 3 but was less effective in Trial 4. Benomyl, thiabendazole and carboxin, each in combination with captan, were also highly effective.

The results of Trial 1 were at considerable variance with those of Trials 2, 3 and 4 in that each fungicide was much less effective. This can be explained if one accepts that the soil of Trial 1 had a high inoculum load against which the tuber treatments were relatively ineffective. This suggestion is reasonable since the soil of Trial 1 was in potatoes the previous year whereas the soil of the other three trials was last in this crop in 1968/69. Particular support for this suggestion is provided by a comparison between Trials 1 and 3 where the seed lines and seed treatments were identical and the planting time and soil type

Table XII Efficiency of fungicides in controlling shoot infections when applied as tuber treatments: Field Trials 1 and 2.

	Emergence (% shoots emerged)	Percentage shoots in each class					Disease Index*
		0	1	2	3	4	
<u>Trial 1</u>							
control	59	21	5	10	36	28	8.17
benomyl	61	17	8	15	28	32	8.32
thiabendazole	87	31	21	15	26	7	4.27
carboxin	95	60	9	12	14	5	2.63
metiram	80	62	0	10	18	10	3.62
captan	80	35	9	14	26	16	5.55
mancozeb	81	23	5	22	33	17	6.62
benomyl- captan	94	73	5	6	14	2	1.87
thiabendazole- captan	73	34	4	16	28	18	6.08
carboxin- captan	83	38	8	8	32	14	5.52
MEMC	59	49	7	16	19	9	3.86
<u>Trial 2</u>							
control	58	31	5	14	33	17	6.30
benomyl	80	93	3	2	2	0	0.29
thiabendazole	83	89	6	3	2	0	0.36
carboxin	82	94	2	2	2	0	0.28
captan	66	92	0	2	4	2	0.76
benomyl- captan	85	94	6	0	0	0	0.06

$$* \text{ Index} = \frac{E(\text{class}^2 \times \text{no. of shoots in given class})}{\text{total no. of shoots per treatment}}$$

Table XIII Efficiency of fungicides in controlling shoot infections when applied as tuber treatments: Field Trials 3 and 4.

	Emergence (% shoots emerged)	Percentage shoots in each class					Disease Index*
		0	1	2	3	4	
<u>Trial 3</u>							
control	75	38	6	8	26	22	6.24
benomyl	93	100	0	0	0	0	0.00
thiabendazole	95	100	0	0	0	0	0.00
carboxin	90	98	1	1	0	0	0.05
metiram	94	86	4	8	2	0	0.54
captan	88	74	6	10	9	1	1.43
mancozeb	90	76	6	6	10	2	1.52
benomyl- captan	90	100	0	0	0	0	0.00
thiabendazole- captan	89	95	4	1	0	0	0.08
carboxin- captan	96	93	2	4	1	0	0.27
MEMC	93	95	4	1	0	0	0.08
<u>Trial 4</u>							
control	74	22	7	17	32	22	7.15
benomyl	100	95	4	1	0	0	0.08
thiabendazole	97	95	2	2	0	1	0.26
carboxin	85	73	6	13	8	0	1.30
metiram	86	51	0	2	35	12	5.15
captan	92	58	9	6	22	5	3.11
mancozeb	84	42	6	13	29	10	4.79
benomyl- captan	98	81	3	8	7	1	1.14
thiabendazole- captan	99	57	17	17	9	0	1.66
carboxin- captan	97	99	0	1	0	0	0.04
MEMC	97	48	19	17	15	1	2.38

$$* \text{ Index} = \frac{E(\text{class}^2 \times \text{no. of shoots in given class})}{\text{total no. shoots per treatment}}$$

very similar, the only major variable being the cropping history of the two soils. Although in Trial 1 some fungicides apparently performed better than others it is not clear whether these differences are a consequence of variation in soil inoculum load or differences in the ability of the fungicides to control soil inoculum.

Emergence figures in all Trials generally reflect the degree of disease control obtained. However, very few actual "misses" occurred in the controls or in any one treatment.

No markedly phytotoxic effects occurred with any treatment although shoots from thiabendazole treated tubers were slower to emerge and less vigorous. This effect was not noted in the glasshouse trials.

Contrary to overseas reports results of the present study indicate that the organic mercury dip treatment of seed tubers as recommended by the manufacturer's (0.5 to 1 minute dip in a solution containing 150 mg/ml a.i.) was highly effective in inactivating sclerotia and in preventing the development of shoot lesions. In Australia it is considered that instantaneous dips are ineffective in the field control of the disease and that treatments with MEMC of at least 15 and preferably 30 minutes are required (Harrison and Downie, 1955; D.E. Harrison and W.A. Downie pers. comm.). Similarly in the Netherlands there is doubt as to the effectiveness of the standard recommendations. According to Emden et al. (1966) -

"it is erroneous to think that seed tubers with many or large sclerotia can be freed completely from infestation by applying the standard disinfection procedures, because these methods are only sufficient to kill mycelium and small sclerotia. In order to disinfect completely seed tubers carrying an appreciable density of sclerotia the prescribed concentration should be at least doubled and the duration of treatment considerably prolonged."

Likewise in the United Kingdom disinfection of seed tubers with the organic mercury fungicides seldom completely eradicates R. solani (Hide et al., 1969; Hirst et al., 1970.)

The results from some overseas investigations on the effect of fungicide dust applications to seed tubers on the development of shoot lesions are summarised in Table XIV and in general agree with those obtained in the present study. However, there is some variability between the overseas tests with the same chemical which can probably be attributed to the varying importance of seed and soil inoculum in each experiment. Presumably these experiments had been designed to obtain control of tuber inoculum since tuber treatments were used, but only Schultz (1966) makes mention of sclerotia on the seed tubers and Schultz (1966) and Davis et al. (1971) are the only investigators who mention previous cropping programmes.

Tuber treatments with specific fungicides could well contribute to the control of other fungal pathogens in one or more of the following ways:

- (a) control of tuber-borne inoculum
- (b) prevention of contamination of clean soils
- (c) control of disease during storage if treatments are applied soon after lifting

Although the fungi in the following list are recorded as potato pathogens in New Zealand (Dingley, 1969), their importance and prevalence is unknown and there would appear to be considerable scope for investigation of these diseases and their control under New Zealand conditions.

<u>Disease</u>	<u>Pathogen</u>
Skin spot	<u>Oospora pustulans</u> Owen and Wakef.
Silver scurf	<u>Helminthosporium solani</u> Dur. and Mont.
Gangrene	<u>Phoma exigua</u> Desm. <u>Phoma foveata</u> Forster
Dry rot	<u>Fusarium</u> spp.
Powdery scab	<u>Spongospora subterranea</u> (Wallr.) Lagerh.
Common scab	<u>Streptomyces scabies</u> (Thaxt.) Waksman and Henrici
Pink rot	<u>Phytophthora erythroseptica</u> Pethybr.
Leak	<u>Pythium ultimum</u> Trow
Wilt	<u>Verticillium albo-atrum</u> Reinke and Berthold <u>Verticillium dahliae</u> Kleb.

Table XIV Effect of fungicide dust treatments on shoot lesioning*:
results of overseas experiments.

	Schultz, (1966)	Jones, (1969)	Cetas, (1969a)	Cetas, (1969b)	Easton, Bailey & Nagle, (1970)	Cetas, (1970)	Cetas, (1971)	Davis, Groskopp & Callihan, (1971)
benomyl			++			++	++	++ - test 1 0 - test 2
thiabendazole					0	++		
carboxin	++		++	++		+		
metiram		0	+			+	+	
captan		0	+			+	++	
mancozeb		0	+			+	++	

*
 0 = No control of stem lesioning
 +) = Intermediate stages
 ++)
 +++ = Total control

To obtain control of some of the above diseases as well as black scurf it may be necessary to use combinations of fungicides, particularly where microbial antagonism is eliminated or suppressed by use of a particular fungicide. For example, since the pythiaceus fungi are insensitive to the benzimidazoles (Bollen and Fuchs, 1970; Edgington et al., 1971) the activities of these fungi may be accentuated with serious effects if benzimidazole fungicides are used alone.

Chapter IV

SYSTEMIC ACTIVITIES OF FUNGICIDES IN POTATO PLANTS

In theory, systemic fungicides would have several advantages over non-systemic fungicides when used as tuber treatments for control of potato black scurf:

1. Protection against soil inoculum by virtue of their systemic uptake.
2. Protection against tuber inoculum not controlled by the tuber dust.
3. Inactivation of shoot infections which occur during storage. Chamberlain (1931a) has shown that when potatoes are stored in damp conditions R. solani can become active, forming sclerotia on shoots and tubers.

Additional benefits from systemic fungicides could result from control of such pathogens as Verticillium albo-atrum Reinke and Berthold, V. dahliae Kleb. and Sclerotinia sclerotiorum (Lib.) de Bary. Verticillium spp. are sensitive to the benzimidazoles (Erwin, Mee and Sims, 1968; Erwin, Sims and Partridge, 1968; Bollen and Fuchs, 1970; Edgington et al., 1971) and carboxin (Edgington and Barron, 1967). S. sclerotiorum is sensitive to benomyl (Pennycook and Corbin, 1970) and currently this fungicide is recommended as a foliar spray for its control in potatoes.

The systemic properties of benomyl, thiabendazole and carboxin in a wide range of plants are well known (Catling, 1969; Weinke, Lauber, Greenwald and Preiser, 1969; Erwin, 1970; Hor, 1972; Kirby, 1972). Claims have also been made for the systemic activity or translocation of captan by Stoddard (1954), Napier, Rhodes, Turner, Tootill and Dunn (1957), May, Palmer and Hacskeylo (1958), Wallen and Hoffman (1959), Rosen (1959), and May and Palmer (1959). However, Fawcett, Spencer and Wain (1958) were unable to confirm the results of Napier et al. (1957), and Somers and Richmond (1962) found only trace amounts of captan in bean leaves after the application of relatively heavy doses to the roots. They suggested that any chemotherapeutic effect of captan cannot be attributed to its systemic activity but probably to some interference with host metabolism. As suggested by Burchfield (1967) it is unlikely that captan is

translocated for any distance in vascular tissue owing to its extremely high instability in aqueous media (Burchfield and Schectman, 1958).

There are no reports known to the writer in which the uptake and translocation or systemic activity of benomyl, thiabendazole, carboxin or captan in potatoes has been investigated. Accordingly the following studies were undertaken.

- A. 1. A preliminary experiment to determine whether benomyl is systemic in potato plants. This experiment involved different planting media and methods of application of the fungicide.
- 2. A trial to determine whether uptake and translocation of benomyl, thiabendazole and captan occurs following dust applications to seed tubers.
- B. An experiment to determine whether fungicides applied as tuber dusts provide systemic protection against inoculum of R. solani added to perlite.

A. UPTAKE AND TRANSLOCATION OF FUNGICIDES IN POTATO SHOOTS

1. Experiment I

Using benomyl as the test fungicide initial studies sought to establish whether uptake and translocation occurred in potatoes planted in soil, perlite, and a blend of peat and perlite. For each planting medium benomyl was applied as a tuber dust, as a drench and by incorporating into the medium, the latter two each at two rates.

An agar plate bioassay was used to reveal whether uptake and translocation had occurred. In this method the fungitoxicant diffuses from tissue pieces into the surrounding seeded agar and inhibits the growth of the test fungus. The amount of fungitoxicant is indicated by the diameter of the resultant zone of inhibition.

- (i) Materials and Methods: Tubers (var. Ilam Hardy) were planted 7 to 10 cm deep at the rate of four per planter bag in three media:
 - (a) a sandy-loam soil, heat treated (80-90C for one hour), pH 6.7
 - (b) a peat:perlite (coarse grade), 1:2 mix, without fertilizer, pH 4.5
 - (c) perlite (coarse grade), without fertilizer, pH 7.8

For each medium there were three methods of application of benomyl:

- (a) A dust (10% a.i.) applied at a rate of 10g dust per kg tubers.
- (b) a drench at rates of 250 ug/ml a.i. and 500 ug/ml a.i. Applications of 1000 ml/planter bag of each suspension were made at seven days and seventeen days after planting.
- (c) the fungicide was uniformly mixed into each medium at rates of 0.3 g a.i. and 0.6 g a.i. per litre of media.

There were thus 15 fungicide treatments, plus three controls (untreated tubers in the three planting media) giving a total of 18 treatments. Two bags (i.e. eight tubers) were used for each treatment. The media were watered at the time of planting and thereafter as required.

Following 31 days growth in a glasshouse (temperature 10-26C) tubers were dug, washed free of the planting media and the shoots detached. Roots and stolons were removed from shoots and the shoots washed thoroughly to remove external fungicide. These shoots were then sterilized by exposure to propylene oxide fumes for 16 hours in sealed bell jars.

The agar plate bioassay method

Penicillium spp. are commonly used as test organisms against benomyl in agar plate bioassays (Erwin, Mee and Sims, 1968; Hine, Johnson and Wenger, 1969; Jacobsen and Williams, 1970; Biehn and Dimond, 1971). The test organism in the present study was a species of Penicillium shown to be highly sensitive to benomyl (Appendix IV).

(i) Preparation of the double strength spore suspension

Cultures were prepared by flooding PDA_L plates with a spore suspension which were then incubated at 24C in the dark for four to five days. This resulted in an even production of spores over the agar surface. The plates were then flooded with sterile water and the resulting conidial suspension shaken vigorously in a McCartney bottle to

disperse the conidia. The suspension was then adjusted to give a final concentration of approximately 40×10^4 conidia/ml (double strength) in 200 ml sterile distilled water. The spore concentration was determined using a Neubauer haemocytometer. Conidial aggregates were each regarded as a "single conidium".

(ii) Preparation of seeded agar plates

The conidial suspension was added to 200 ml of double strength PDA_L held at 50-55C and after thorough mixing the seeded agar was added to petri dishes (17 ml per dish) and allowed to solidify. The plates were stored in a refrigerator until used, usually within three hours.

(iii) Bioassay of tissue pieces

Three transverse sections (4 mm long) were cut from each sterilized shoot at the following locations:

- (a) a section 0.5 to 1.0 cm above the base of the shoot.
- (b) a section from the middle of the shoot.
- (c) a section from the margin of green and white tissue (the soil line approximately).

With the aid of a cork borer a leaf disc (4 mm diam.) was also removed from the margin of one of the younger leaves of each shoot.

All tissue pieces were then placed on the seeded agar plates and gently smoothed down to ensure good contact between the tissue and the agar surface. Four tissue pieces (three sections and one disc) were placed on each plate. That is, one total shoot was assayed per plate. Ten shoots were assayed for each treatment.

To determine whether fungicide had penetrated seed tubers, tissue from these tubers was plated to seeded agar. A core was removed from two tubers of each treatment with a cork borer (7 mm) and the outer 1 mm cap removed to avoid any effects of external fungicide. Transverse sections (4 mm long)

were cut from several locations on these cores and plated to seeded agar.

All plates were incubated at 24C in the dark for three days.

(iv) Recording of results

The results were recorded on the third day of incubation by averaging two measurements of the inhibition zone taken at right angles to each other. Only the size of the clear zone was recorded, the intervening narrow annulus of partial fungal growth being ignored. The diameter of each shoot section was deducted from the inhibition zone diameter to obtain the effective diameter of the zone of inhibition. The average effective diameter of the inhibition zone of all replicates was used for comparison between treatments.

The quantity of benomyl in plated tissues may be estimated by reference to the standard curve in Appendix IV. The curve shows the relationship between the quantity of benomyl and the average effective diameter of a zone of inhibition.

- (ii) Results and Discussion: The results are presented in Figures 16 and 17 and indicate that benomyl, or probably a fungitoxic derivative, MBC* (Clemons and Sisler, 1969; Peterson and Edgington, 1969; Sims, Mee and Erwin, 1969; Peterson and Edgington, 1970, 1971) became systemic with all treatments. However, the extent of uptake depended a great deal on the planting medium used and the method of application of benomyl.

For all methods of application the amount of fungitoxicant was greatest in shoot sections of plants grown in perlite. Considerably smaller zones of inhibition were produced from shoot sections from the soil and peat:perlite series. This agrees with the

* methyl 2 - benzimidazolecarbamate

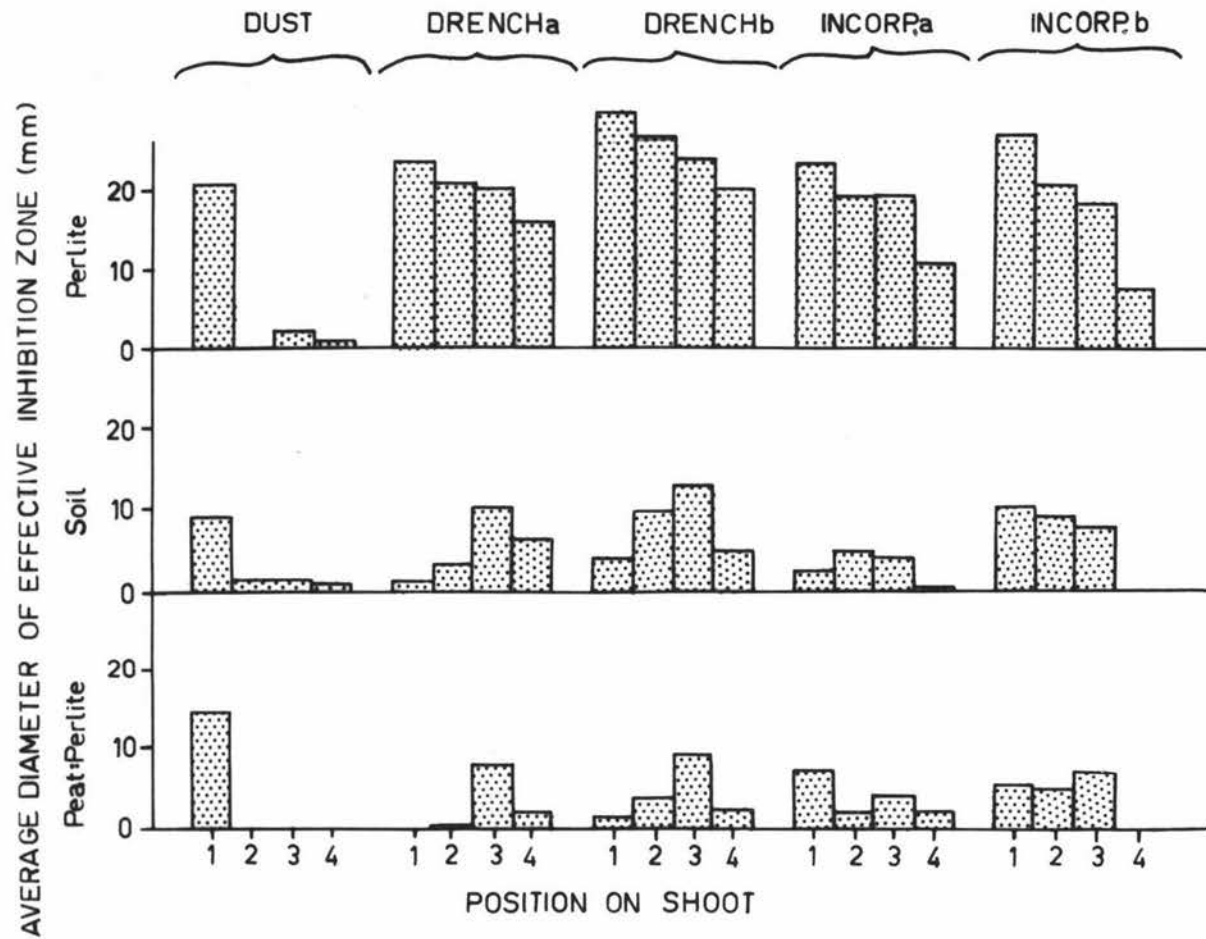
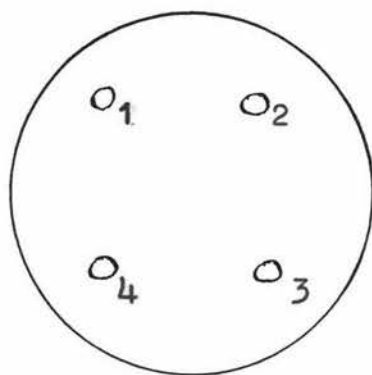
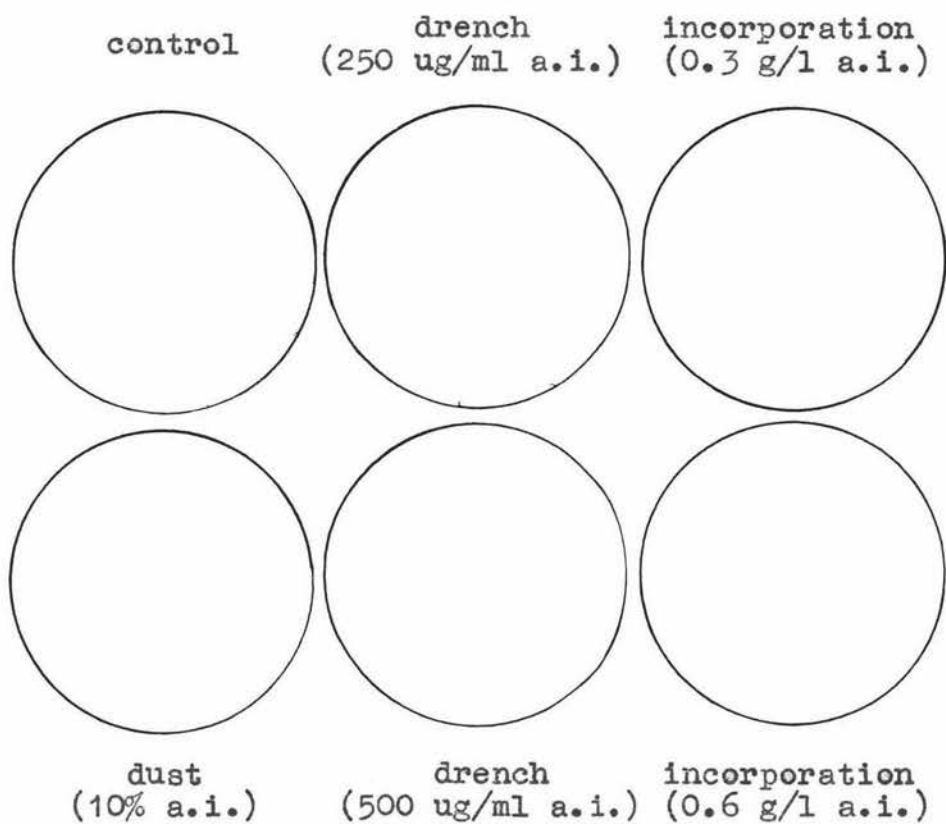


FIGURE 16 Effect of planting medium and method of application of Benomyl on the distribution of Fungitoxicant in potato shoots.

Figure 17 Inhibition zones produced by different parts of potato shoots on Penicillium-seeded agar following treatments involving different planting media, and methods and rates of application of benomyl.

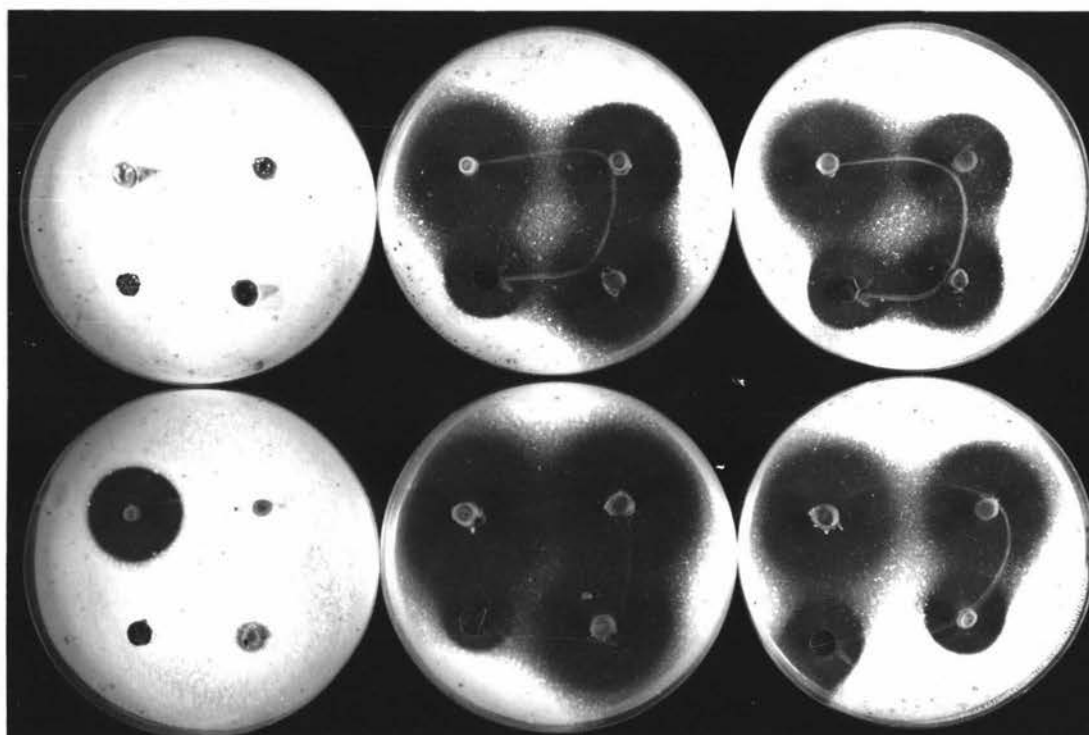
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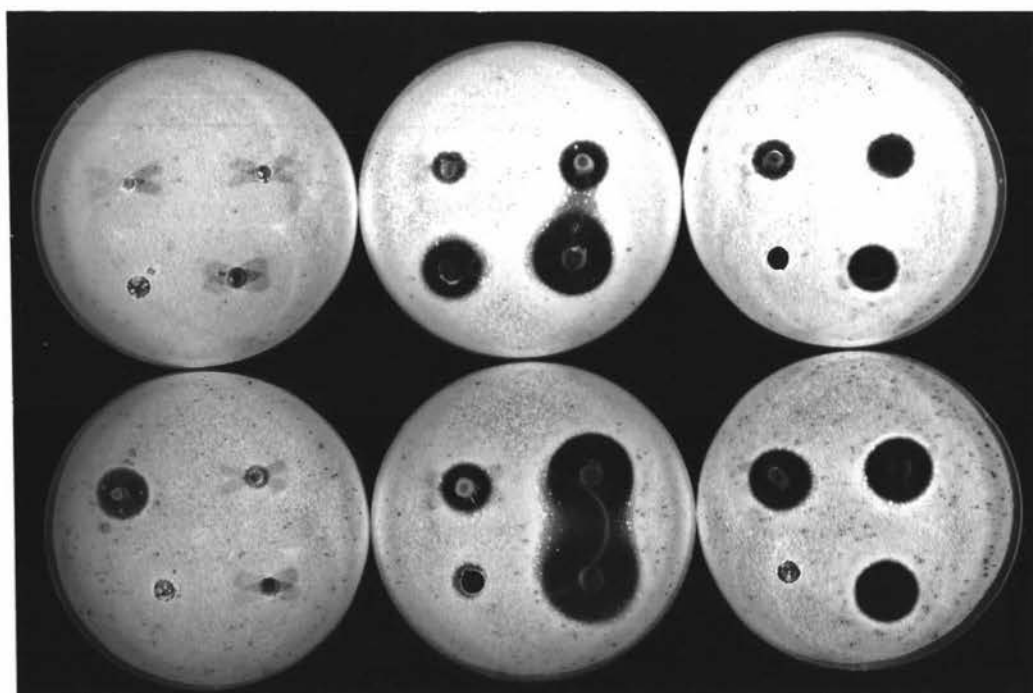
Tissue piece from:

1. base of shoot
2. middle of shoot
3. margin of green and white tissue
4. leaf (disc)

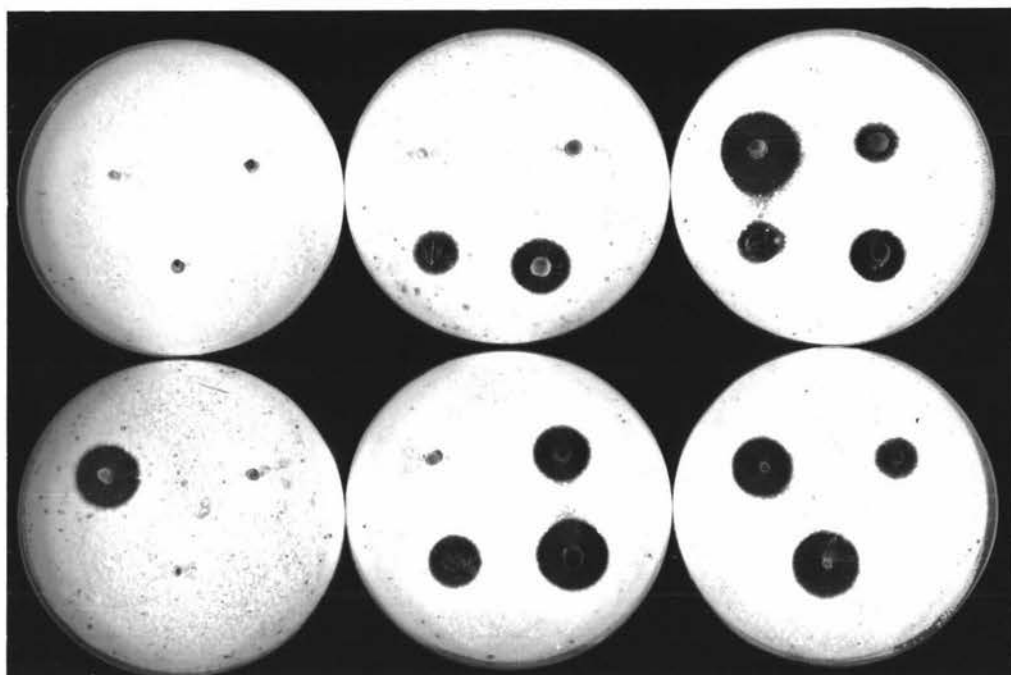
- A. Perlite
- B. Soil
- C. Peat:perlite



A. Perlite



B. Soil



C. Peat:Perlite

findings of other investigators (Hine et al., 1969; Zaronsky and Stipes, 1969; Hock, Schreiber and Roberts, 1970; Pellissier, Lacasse and Cole, 1971; Schreiber, Hock and Roberts, 1971) who have shown that greater uptake of benomyl occurs from media low in organic matter. It is surprising that such little uptake occurred from the soil used in this experiment since it was low in organic matter.

In both perlite and soil slightly greater uptake of fungitoxicant occurred with drench applications than with media incorporation. Conversely in peat:perlite greater uptake resulted from media incorporations. In all media the increased application rate (both drench and incorporation) resulted in larger zones of inhibition.

As regards the distribution of the fungitoxicant in shoots, there was a progressive decrease in the amount from the base of shoots to the leaves in the perlite series where benomyl was applied both as a drench, and incorporated into the perlite. However, in the soil and peat:perlite series distribution of fungicide was more erratic.

Where seed tubers were dusted with benomyl zones of inhibition of significant size developed only around those sections removed from the base of shoots, indicating limited uptake of fungicide. This was the case with all media.

No zones developed around plugs of tuber tissue from any of the treatments showing that fungicide did not penetrate the tuber skin.

2. Experiment II

In this experiment the uptake and translocation of benomyl, thiabendazole and captan in potato shoots following dust applications to tubers planted in both soil and perlite was investigated using the agar plate bioassay method. The test organism used was a species of Penicillium highly sensitive to benomyl and thiabendazole, and to a lesser extent sensitive to captan (Appendix IV).

- (i) Materials and Methods: Tubers were treated at a rate of 10 g 10% a.i. fungicide dust per kg of tubers and planted in soil (sandy loam, heat treated (80-90C for one hour), pH 6.7) or perlite (pH 7.8). The plants were harvested after 31 days and 10 shoots per treatment assayed according to the procedure outlined in Experiment I.
- (ii) Results: The results presented in Figures 18 and 19 show that there was very little uptake of benomyl or thiabendazole beyond the basal regions of shoots. Zones of inhibition were slightly greater from sections removed from shoots grown in perlite than from those grown in soil. The results of this experiment for benomyl agree with those obtained in Experiment I.

No zones of inhibition were obtained from sections removed from shoots developing from captan treated tubers, indicating this fungicide does not become systemic in potato plants.

The above experiment was repeated with essentially similar results.

3. Conclusion

It would seem that there is little benefit to be expected from the systemic properties of benomyl and thiabendazole when applied as dusts to whole tubers since uptake was limited to the basal regions of shoots. Reasons for this limited uptake are not readily apparent. However, there would seem to be three possibilities:

- (i) Since uptake and accumulation of fungicide is dependent on transpiration (Peterson and Edgington, 1971; Kirby, 1972) it is possible that lack of transpiration was responsible. However, in the foregoing experiments plants were up to 7 to 10 cm high, particularly when grown in soil, thus allowing for considerable transpiration. Further, when applied as a drench to perlite uptake of benomyl was considerable, which suggests that the method of application rather than

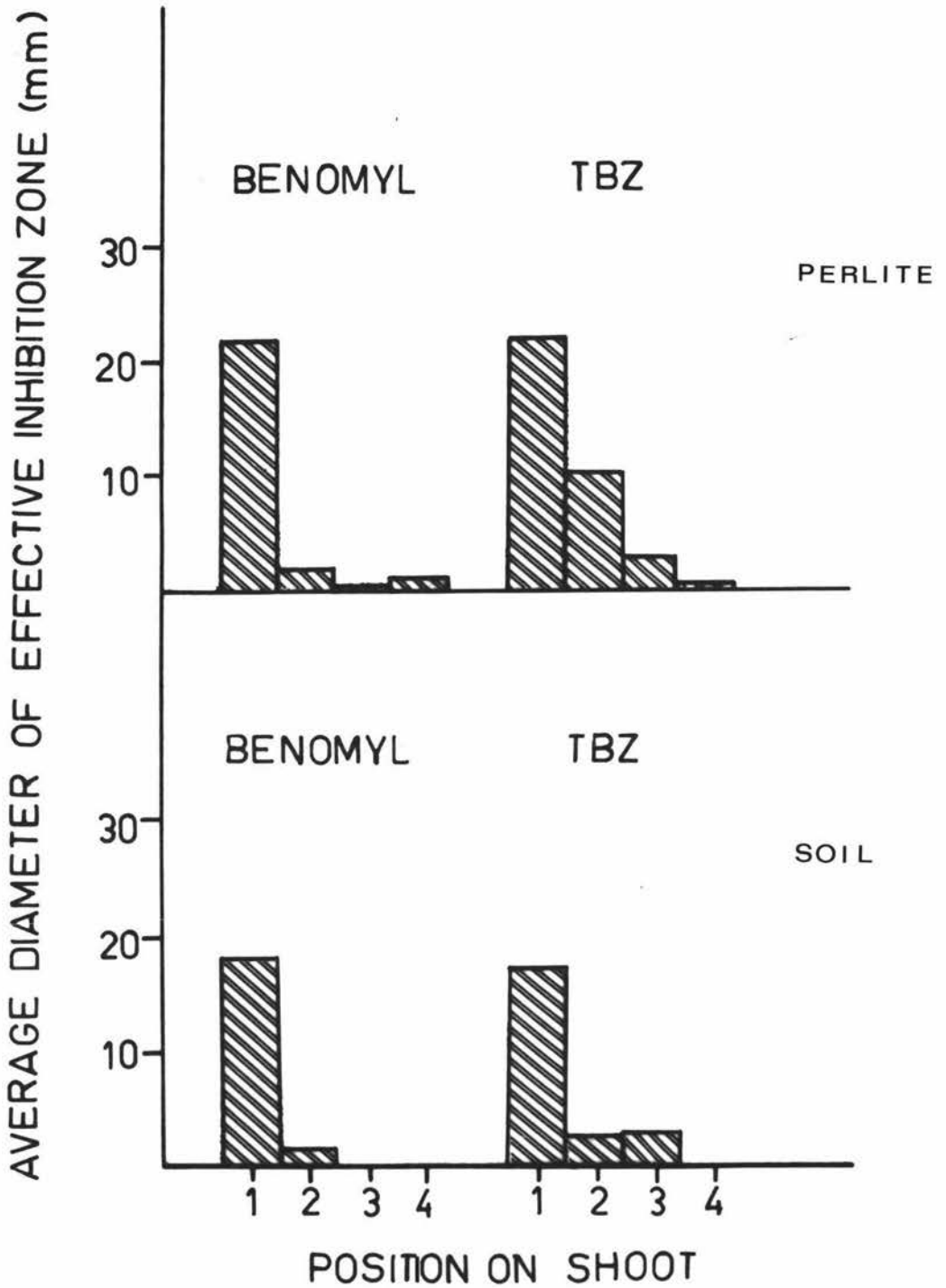
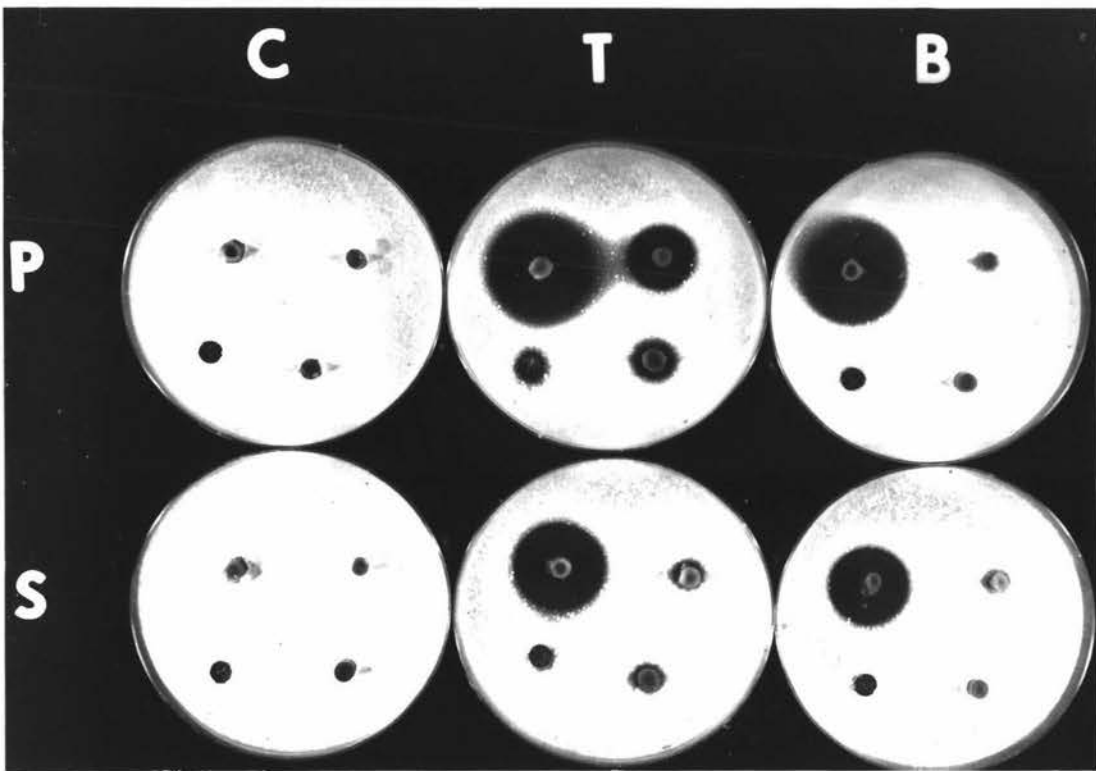
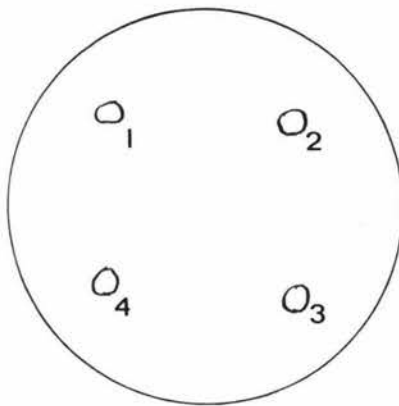


FIGURE 18 Distribution of Fungitoxicant in potato shoots produced from tubers dust treated with Benomyl or Thiabendazole and planted in soil or Perlite.



P - Perlite
S - Soil

C - control
T - thiabendazole
B - benomyl



Tissue piece from:
1. base of shoot
2. middle of shoot
3. margin of green and
white tissue
4. leaf (disc)

Figure 19 Inhibition zones produced by different parts of potato shoots on Penicillium seeded agar following dust applications of benomyl and thiabendazole to seed tubers planted in perlite or soil.

transpiration itself was responsible for the limited uptake.

- (ii) When fungicides are applied as tuber dusts, insufficient chemical was available in the root zone of shoots to allow for substantial uptake.
- (iii) It is possible that, rather than being taken up by roots, the fungicides were absorbed directly by shoots and hence zones of inhibition were produced only from sections removed from the basal regions of shoots.

It should also be noted that in these experiments shoots were assayed at a specific point in time and the results give no indication of when uptake actually occurred. It could be that the limited uptake will be of no benefit because it does not occur soon enough.

C. SYSTEMIC ACTIVITY OF FUNGICIDES AGAINST R. SOLANI

One method of determining the systemicity of a chemical is to place it at a position on the plant remote from where disease expression occurs (Erwin, 1969). There is some difficulty in doing this with a disease such as black scurf. However, since captan, benomyl, thiabendazole are non-volatile and relatively insoluble in water (Martin, 1971) it was thought that presumptive evidence for or against systemic activity of these fungicides could be obtained by inoculating planting media in which dust treated tubers were grown.

1. Material and Methods: Inoculum-free seed was obtained by selecting tubers (var. Ilam Hardy) with very few sclerotia, removing as many of the sclerotia as possible, and dipping the tubers in the recommended concentration of MEMC (150 ug/ml a.i.) for two minutes. Groups of 75 tubers were then treated with dusts of benomyl, captan, thiabendazole (each 10% a.i.) at a rate of 10g fungicide dust per kg tubers. Untreated tubers served as controls. The tubers were planted 7 to 10 cm deep in coarse grade perlite at the rate of five tubers per planter bag.

Six days later plants were inoculated by pouring macerated agar cultures of R. solani over the perlite. The

isolate used was obtained from lesioned potato shoots and grown on PDA_L in petri plates (9 cm) for seven days, after which the cultures were macerated in tap water with a Waring blender. Three hundred millilitres of the resulting suspension were poured over each bag followed by 300 ml of tap water. Three inoculum levels were used:

- (a) one half of a 9 cm agar plate culture per bag
- (b) one and one-third agar plate cultures per bag
- (c) two agar plate cultures per bag

Seventy five tubers of each fungicide treatment were inoculated in this way: that is, twenty-five tubers for each inoculum level. Twenty-five tubers were left uninoculated as controls.

At seven days and thirteen days after inoculation each bag was watered with 300 ml of a potato broth* to provide a food base for the fungus.

After 29 days growth plants were dug, washed and the shoots assessed for disease damage. Disease indices were also calculated as for the glasshouse trials (p. 56).

2. Results and Discussion: The results are presented in Table XV. Uninoculated plants produced disease-free shoots, indicating the effectiveness of the mercury dip treatment in inactivating tuber-borne inoculum, which in turn meant that infections developing on shoots of inoculated plants originated from inoculum added to the perlite.

In all treatments the severity of disease progressively increased with each increase in inoculum level. Compared with the non-dusted controls, the fungicides did not control the disease at the lowest inoculum level. However, some reduction in disease severity was effected by benomyl, thiazobenzazole and carboxin at the two higher inoculum rates. These results are regarded as providing presumptive evidence for some systemic activity of these fungicides against inoculum present in the planting medium.

* Prepared by boiling 2.5 kg of potatoes in two litres of water for one hour and diluting the resulting extract 10 times with tap water.

The limited control obtained was not surprising in view of the agar plate bioassays in Experiments I and II (Section A) showing that uptake of benomyl and thiabendazole was limited and largely confined to the base of shoots.

Since in the above experiment inoculum was added to the perlite six days after planting, there was every opportunity for these fungicides to provide systemic protection against R. solani. In a field situation, however, soil-borne inoculum would be present at the time of planting, which may mean that infections could occur before the fungicides were taken up into shoots.

The results failed to provide evidence that captan has systemic activity in potato plants. This was not unexpected since no uptake and translocation could be demonstrated in the agar plate bioassay of Experiment II (Section A).

Table XV Efficiency of fungicides applied as tuber dusts in controlling inoculum of R. solani added to the planting medium (perlite).

	INOCULUM LEVELS																	
	a (lowest)					Disease* Index	b					Disease* Index	c (highest)					Disease* Index
	percentage shoots in each class						percentage shoots in each class						percentage shoots in each class					
0	1	2	3	4	0	1	2	3	4	0	1	2	3	4				
control	62	9	10	14	5	2.5	5	3	4	37	51	11.7	4	0	1	11	84	14.5
captan	71	0	10	12	7	2.6	23	0	0	8	69	11.8	4	0	0	2	94	15.2
benomyl	69	6	7	10	8	2.5	37	4	8	17	34	7.3	18	0	0	23	59	11.5
thiabendazole	62	8	12	9	9	2.8	26	4	8	20	42	9.0	17	2	9	34	38	9.5
carboxin	71	4	4	11	10	3.0	42	5	6	27	20	5.9	33	1	5	21	40	8.5

* Index =
$$\frac{E(\text{class}^2 \times \text{no. of shoots in a given class})}{\text{total no. shoots per treatment}}$$

APPENDIX ICOMPOSITION AND PREPARATION OF CULTURE MEDIA

The media were prepared as described below and in all cases were sterilized by autoclaving at 15 p.s.i. for 20 minutes.

(i) Laboratory potato-dextrose agar (PDA_L)

agar (Davis)	12 g
potatoes (peeled and sliced)	200 g
dextrose	10 g
distilled water	1,000 ml

The sliced potatoes were cooked gently for one hour in 500 ml of distilled water, after which the filtrate was obtained by straining through cheesecloth. The agar and dextrose were melted in 500 ml of distilled water and the potato filtrate then added.

(ii) Difco potato-dextrose agar (PDA_D)

potato dextrose agar (Difco)	39 g
distilled water	1,000 ml

The potato-dextrose agar was soaked in the distilled water for approximately 15 minutes before autoclaving.

(iii) Water agar (WA)

agar (Davis)	12 g
distilled water	1,000 ml

The agar was melted in 500 ml of distilled water and the solution made up to 1,000 ml. Penicillin and streptomycin (each at 50 ug/ml) were added after autoclaving when the agar had cooled to approximately 50C.

APPENDIX IIISOLATION AND IDENTIFICATION OF R. SOLANI

The following procedures were employed in those experiments where it was necessary to confirm that R. solani was in fact the fungus involved.

A. ISOLATION

Diseased material (shoots, roots, stolons) was washed thoroughly in running tap water for several minutes to remove associated debris and soil, and dried between clean filter papers. Tissue pieces were cut with a flamed scalpel from the margins of healthy and diseased tissue and plated to water agar containing 50 ug/ml each of streptomycin and penicillin. Following incubation for two days at 24C in the dark, hyphae of R. solani had usually emanated from the tissue pieces. The growth rate of R. solani was sufficiently rapid to outgrow fungal and/or bacterial contaminants and pure cultures of the fungus could be obtained by transferring an agar plug to FDA_L slopes or plates.

B. IDENTIFICATION

Hyphae were examined under a compound microscope (x100) and isolates identified as R. solani on the basis of the following criteria (Parmeter and Whitney, 1970).

1. Branching near the distal septum of cells in young hyphae.
2. Constriction of the branch and formation of a septum in the branch near the point of origin.

These two characteristics are illustrated in Figure 20.

Two further criteria, although lacking in some isolates of R. solani (Parmeter and Whitney, 1970), were also used.

1. Hyphae greater than 5u in diameter.
2. Rapid growth rate on culture media.

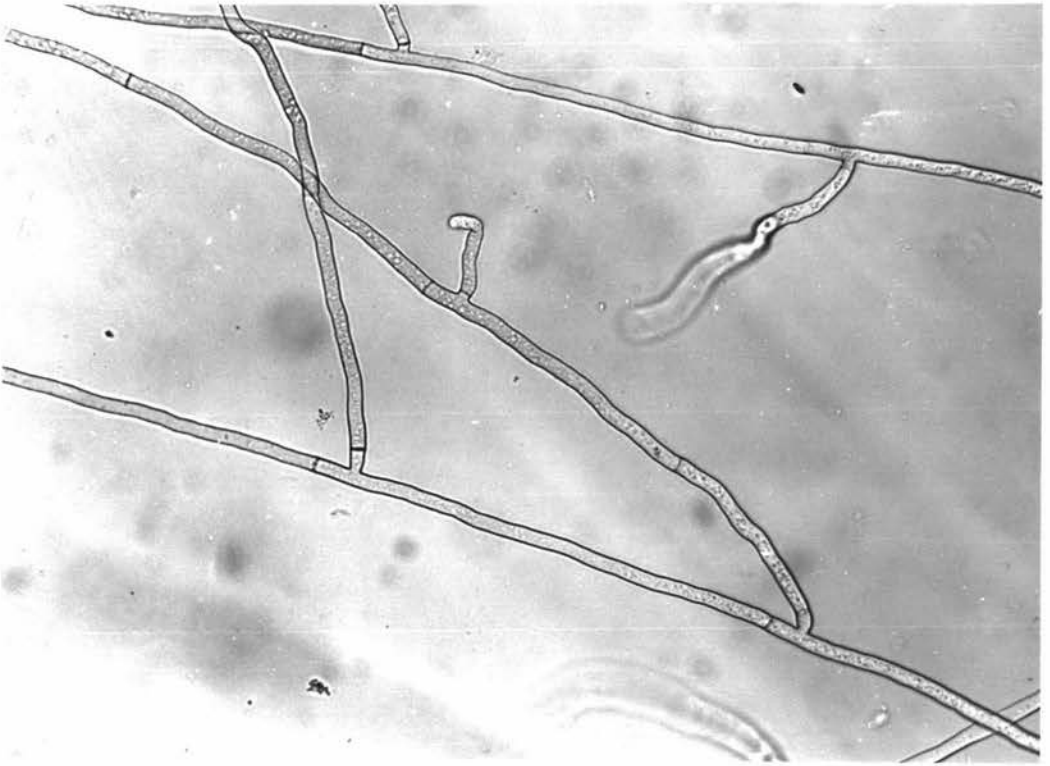


Figure 20 Hyphae of *R. solani* on WA showing characteristic branching (x 200).

APPENDIX IIISCREENING OF SEED LINES FOR PATHOGENIC R. SOLANI

To determine to what extent pathogenic strains of R. solani were present on certified seed tubers, 23 lines (7 varieties) in 1971 and 26 lines (11 varieties) in 1972 were screened as follows.

Twenty tubers of each sample were planted 10 to 13 cm deep in perlite in planter bags (Figure 21). For practical purposes perlite may be regarded as being free of R. solani which implies that any disease development must have arisen from tuber-borne inoculum. Five tubers were planted per bag and the perlite watered at planting time and subsequently as required.

After four to five weeks growth (when plants had emerged) the tubers were dug and the percentage of shoots with lesions recorded for each seed line. Frequent isolations from lesioned shoots (Appendix II) confirmed R. solani as the pathogen involved.

The results (Tables XVI and XVII) show that pathogenic strains of R. solani were present on all seed lines examined, indicating seed tubers as a consistent source of inoculum.



Figure 21 Planter bags used for seed screening. Potatoes after three weeks growth in perlite.

Table XVI Incidence of pathogenic R. solani
on certified potato seed lines:
1970-71

Line	Variety	Origin	Percentage Shoots with lesions
1	Ilam Hardy	South Island	90
2	" "	" "	93
3	" "	" "	84
4	" "	" "	95
5	" "	" "	93
6	" "	" "	85
7	" "	" "	85
8	" "	" "	100
9	" "	" "	64
10	" "	North Island	97
11	" "	" "	88
12	" "	" "	91
13	Rua	South Island	63
14	"	" "	67
15	"	North Island	76
16	"	" "	75
17	"	" "	97
18	"	" "	84
19	Sebago	South Island	95
20	Glen Ilam	" "	70
21	Red King Edward	" "	87
22	Kurrel	North Island	98
23	Toru	" "	80

Table XVII Incidence of pathogenic *R. solani*
on certified potato seed lines
1971-72

Line	Variety	Origin	Percentage Shoots with lesions
1	Ilam Hardy	South Island	94
2	" "	" "	78
3	" "	North Island	66
4	Rua	South Island	46
5	"	" "	76
6	"	" "	46
7	"	" "	26
8	"	North Island	48
9	"	" "	21
10	"	" "	44
11	"	" "	36
12	"	" "	18
13	"	" "	37
14	"	" "	38
15	Sebago	" "	82
16	"	" "	9
17	Glen Ilam	" "	75
18	" "	" "	67
19	Aucklander Short Top	" "	64
20	" " " "	South Island	55
21	Kurrel	North Island	38
22	Rima	" "	64
23	Ono	" "	54
24	Katahdin	South Island	53
25	Dakota	" "	88
26	Pentland Dell	" "	36

APPENDIX IVSTANDARDISATION OF THE DIAMETER OF THE
INHIBITORY ZONE PRODUCED BY KNOWN
QUANTITIES OF FUNGICIDE

This experiment was conducted to enable estimation of the quantity of fungitoxicant present in shoot tissue of treated plants, and also to determine the sensitivity of the test fungus to benomyl, thiabendazole and captan.

1. Materials and Methods

Penicillium seeded agar plates were prepared, as previously described (p. 79). Suspensions of benomyl, thiabendazole, and captan at 0, 5, 10, 25, 50, 100, 500 ug/ml a.i. were prepared in 20 ml of sterile distilled water from stock solutions containing 500 ug/ml a.i.

For each concentration of fungicide six wells were cut in the seeded agar plates with a seven millimetre cork borer. The number of wells per plate varied between one and four. With the aid of a pipette 0.1 ml of fungicide suspension of the appropriate concentration was added to each well. The plates were then incubated at 24C in the dark for three days.

The diameter of the zone of inhibition was measured by averaging two measurements at right angles to each other. Only the middle clear zone was recorded, the intervening narrow annulus of partial fungal growth being ignored. To obtain the effective zone of inhibition the diameter of the well (7 mm) was deducted from the diameter of the zone measured. The average effective diameter of the inhibition zone of all replicates was used for comparison between treatments.

2. Results and Discussion

The results presented in Table XVIII and Figures 22 and 23 indicate that the fungus was sensitive to all three fungicides and therefore suitable for use as a test organism. However, benomyl and thiabendazole were more effective than captan in inhibiting growth of the fungus, with thiabendazole slightly more effective than benomyl.

Increasing the quantity of fungicide added to wells produced a progressive increase in the diameter of inhibition zones. Thus the quantity of fungitoxicant in tissue pieces can be estimated by using the curves in Figure 22. However, diffusion of fungicide into agar from wells may differ from diffusion from tissue pieces, thus preventing precise calculations of the amount of fungicide in plant tissue.

Table XVIII Effective zone of inhibition produced by different quantities of benomyl, thiabendazole and captan on plates seeded with a Penicillium sp. and incubated for three days at 24C.

Concentration of fungicide used (ug/ml a.i.)	Equivalent amount of fungicide (ug/7mm well)	Average diameter effective inhibition zone (mm)		
		benomyl	thiabendazole	captan
0	0	0	0	0
5	0.5	11.0	6.0	Trace
10	1.0	20.0	12.1	Trace
25	2.5	25.3	30.1	5.0
50	5.0	27.0	32.0	10.0
100	10.0	29.0	35.0	21.0
500	50.0	37.0	39.0	25.0

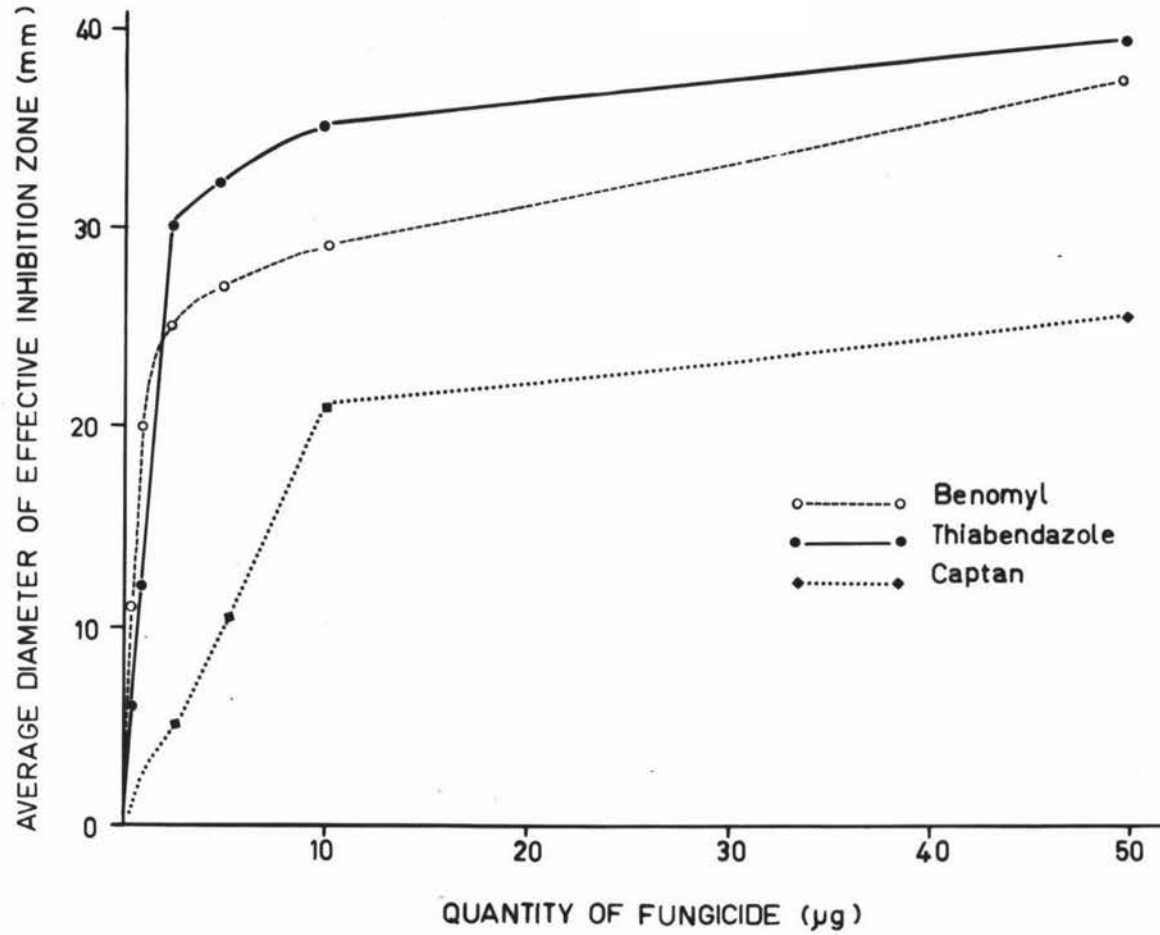


FIGURE 22 Correlation between the quantity of Benomyl, Thiabendazole and Captan and the average effective diameter of the inhibition zone produced on Penicillium-seeded PDA_L plates incubated for three days at 24 °C.

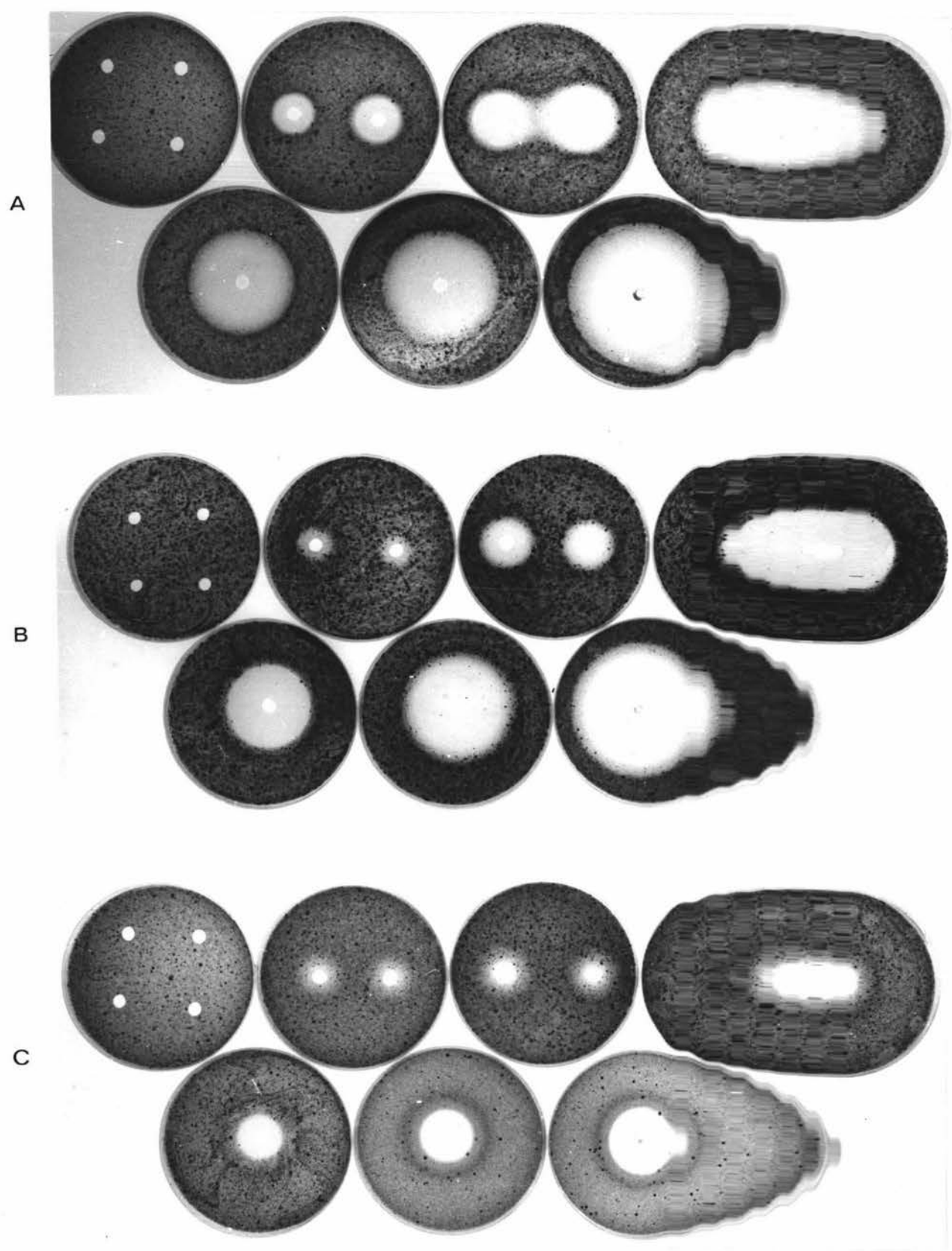


Figure 23 Inhibition zones produced by known quantities of fungicides. Top row, left to right, wells contain 0, 0.5, 1.0, 2.5 ug a.i.; bottom row, left to right, wells contain 5.0, 10.0, 50.0 ug a.i.
 A. benomyl
 B. thiabendazole
 C. captan

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